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Putting Europe's missiles at risk

Next year will be a bad year for European governments, perplexed by strategic problems and bothered by elections. The United States should help them out.

1983 promises to be a rowdy year for nuclear strategists, a testing time for the governments of states belonging to the North Atlantic Treaty Organization (NATO) and one of the most strenuous years for NATO itself since the row about the European Defence Force early in the 1950s and the departure of France at the end of that decade. Although the new year is still some weeks off, the pressure is already building up, principally about the decision taken unanimously by the NATO governments at the end of 1979 to deploy new intermediate range nuclear weapons in Western Europe towards the end of 1983. The plan is that 108 Pershing II ballistic missiles should be sited in West Germany, and that 362 ground-based cruise missiles should be based in West Germany, Italy, the Netherlands and the United Kingdom.

Predictably, the Soviet Union is doing everything it can to frighten governments in Western Europe with the consequences of their decision three years ago. Soviet spokesmen have taken to saying that the short flight-time of the Pershing missiles might force Soviet forces onto the hair-trigger strategy of "launch-on-warning", with all the dangers of accidents that would follow. There have also been heavy hints that it may be necessary to install more of the 5,000-kilometre SS-20 mobile missiles on the Soviet side, that sea-launched cruise missiles might be placed within range of the United States and even that ballistic missiles might again be based in Cuba. The Soviet Union's obvious objective is to undo the 1979 agreement. It is high time that Western governments worked out a resolute response.

The present, unfortunately, is an unpropitious time for working out a level-headed response. Of the principal governments involved in the 1979 decision, only the British government has not been changed, either by the ballot-box or by some other constitutional process. And while it might in ordinary circumstances have been expected that the new West German government of Chancellor Helmut Kohl would have been more able politically to carry through the 1979 agreement than its predecessor, haunted as it was by disagreements on nuclear policy within its own party, Chancellor Kohl is almost as much constrained by his promise to hold a general election next March. Elsewhere in Europe, even the strongest governments have been given pause by the resurgence of opinions dissenting from present strategies. The present British government, for example, has to contend with — and ideally must somehow answer — an increasingly vocal Campaign for Nuclear Disarmament that would abandon the plan to replace the British Polaris submarines with Tridents, decline to provide bases for US cruise missiles and eventually (but not yet) withdraw from NATO, a Labour opposition party now committed to the abandonment of Trident and even an opinion from a Church of England group tending in the same direction. What, in these circumstances, is to be done?

The first need is that embattled governments should remind their people of the reasons for the 1979 agreement and of its terms. The decision to base missiles of intermediate (as distinct from "tactical") range in Western Europe derives simply from the deployment of the Soviet force of SS-20s during the 1970s. With the help of the earlier SS-5 and SS-6 missiles, the Soviet Union could now deliver more than 1,000 warheads on targets in Western Europe using delivery systems that can have no wider strategic role. (The mobile SS-20s are also potentially a threat to

China, but most of them just now are based in the central and western parts of the Soviet Union.) To be sure, both sides could carry larger numbers of nuclear warheads to targets some thousands of kilometres away by means of aircraft, but plainly there are technical advantages in a force of ballistic missiles and in the diversity that they bring. If some measure of strict military balance is a legitimate goal of strategic policy, the NATO decision of 1979 was almost unavoidable. On the view, for which there is a rich body of argument in justification, that the nuclear balance has helped to keep the peace in Europe in the past several decades, the decision may also be held to have been prudent.

What has been commonly overlooked, in the arguments between Western governments and their nuclear critics, is that NATO's decision in 1979 was deliberately conditional. The governments concerned said loudly and clearly that intermediate range missiles would be based in Western Europe by the end of 1983 only if, by then, there had been no substantial progress towards arms control in the European region. At the time, the West German government was chief among those that argued that no intermediate range missiles on either side would be preferable to other solutions.

The experience of the past several months at the Intermediate Nuclear Force negotiations (INF) at Geneva is far from encouraging. So far, the United States has publicly proposed that all intermediate-range missiles should be dismantled, while the Soviet Union has countered in the private meetings with the suggestion that missile launchers should be limited to 300 on each side. Neither of these proposals can be expected to satisfy the other side, especially when account is taken of the complications arising from the substantial fleets of aircraft on each side, and the presence of independent strategic nuclear forces in both Britain and France.

The gap between the two views is not, however, unbridgeable. Technically, the urgent need is for some means of assigning potency to delivery systems of various kinds, and then for settling on appropriate upper limits that should not be exceeded. The political ideal would be to persuade the Soviet Union to withdraw its SS-20s from within striking distance of Western Europe (perhaps to some observable quarantine in Eastern Siberia?) so that the deployment of the Pershing and cruise missiles could be postponed. But that objective entails a paradox: the most effective way of winning an understanding with the Soviet Union is to continue to insist that the deployment of the US missiles will be carried through. On past form, a compromise should then be possible about the middle of 1983.

Long before then, the Geneva talks must be put on a much more rational basis. Two sets of bilateral negotiations, about nuclear missiles of intermediate and strategic range, are being carried on independently of each other. The distinction is absurd. The SS-20s that now potentially threaten Western Europe could be used both in a limited nuclear war in which the United States was not directly attacked (or threatened) or in a strategic all-button conflict. Similarly (Soviet analysts will calculate), Pershings deployed in Europe would be assigned to a variety of targets in the Soviet Union, some of which would necessarily be integral to a strategic attack (no doubt retaliatory), which probably explains why the Soviet Union has reacted so strongly against them. In such a negotiation, the British and French



governments should either be participants or effectively be represented.

For Western governments, the implications of this complicated situation are bound to be uncomfortable. In Europe, governments will have to be telling anyone who will listen that they stand by what was agreed in 1979, but that they will refuse house-room for the Pershing and cruise missiles once there is some kind of agreement at Geneva. They will have to give further hostages to electoral fortune by pointing out the inconsistencies in the Soviet Union's insistence that it must be allowed to negotiate from strength. In the United States, however, there is an urgent need that the Reagan government should change tack. More factual and less strident accounts of the Soviet threat would help the Helmut Kohls of this world, while the eagerness of members of the US Administration such as Mr Caspar Weinberger, the Secretary of Defense, to prove that the Soviet Union is "not serious" about arms control is increasingly an impediment to a successful negotiation. Western governments in 1983 should try to persuade their own people that they are equally serious in their intentions both to increase the nuclear striking power in Europe and to negotiate a deal with the Soviet Union.

Max Born's centenary

*Quantum mechanics is less than a century old;
Max Born belongs to an earlier period.*

The accompanying photograph of the late Professor Max Born is a monument to the heroic days of modern physics. Born was born almost exactly a century ago, and his anniversary has been widely celebrated in West Germany in the past few months, the fact of his having been driven out in the 1930s notwithstanding. Max Born is still widely revered as a perceptive and sympathetic teacher, as well as the author of an important textbook on atomic physics. Some may also know that with Jordan and, later, with Heisenberg, he contributed to the construction of quantum mechanics, still the most surprising theoretical construct of the twentieth century. Born's achievement, in the early 1920s, was that of reconciling in his own mind the notion that quantum states can be likened to vectors in some n -dimensional or even infinitely dimensional space, and physical quantities to operators in that



space, a triumph of logic over intuition. That Schrödinger eventually stole the show was a kind of accident. And, in any case, Max Born eventually taught physics at the University of Edinburgh as if he had never been a hero.

Can there be excess TV?

Britain has changed from having too little to having too much television.

A few weeks ago, the British were widely regarded as a televisually deprived people, stuck with a meagre ration of three television channels, two run by the British Broadcasting Corporation and one other operated on behalf of commercial interests by a public corporation called the Independent Broadcasting Authority (IBA), financed by those given a licence to broadcast television signals accompanied by advertisements. The late Lord Thomson's remark that a television franchise was a licence to print money has ever since stuck in the public mind and has also been a challenge to the statutory broadcasting authorities: how can the entrepreneurs be kept in their tradesmen's places (chairmen of commercial television companies do not automatically qualify for knighthoods in the British system) while being cajoled into producing better television broadcasts than those that the marketplace would require?

The simple answer, so far, has been to let them make profits, but to take away the cream by means of a levy over and above taxation. More recently, IBA has required that profitable television contractors should contribute towards the cost of an extra television channel. Now, however, the broadcasting authorities are in a fix. The British government, more by accident than design, has decreed not merely that there should be at least two and possibly four extra television channels by 1986, but that there should be a national cable television system even sooner. And then, further to complicate the relief of visual deprivation, the British government has launched a fourth conventionally broadcast set of television signals among the British people, called Channel 4 by English-speaking people, but *Canl Pedwar Cymru* among those in Wales who are able, linguistically, to insist on speaking Welsh.

The immediate (but admittedly anecdotal) consequence of this development has been that people recently crying for more televisual choice have taken to complaining that they cannot possibly keep up with the output of four channels. Merely reading the menus on offer each day is a sufficient chore; actually watching what is on offer would be death of a kind. This, no doubt, is the reason why Channel 4 has so far been a disaster. It seems to have won the allegiance of a mere four per cent of the British televiewing audience, chiefly (it appears) on the strength of ancient films from Hollywood. What the new channel has so far failed to accomplish is the trick of making what it has to show overwhelmingly captivating for some minority among that section of the population at risk of having no other television signal at which to look. Why, in other words, has not the British Channel 4, the first national television network anywhere to be known by a number greater than three, failed in its first few weeks to live up to the ideals of its prospectus? Because it has not had the courage of its conviction that minority interests should be catered for. Science, for example, has been dealt with shabbily on the fourth television channel. On present form, the sixty-fourth channel will be no better.

The moral for the British broadcasting authorities, the British Broadcasting Corporation and the Independent Broadcasting Authority, is that they should practise what they preach, and in particular agree that as the opportunities for people to choose what they watch expand, the need that statutory authorities should oversee what people watch will inevitably decline. It is understandable but nonetheless unforgivable that the broadcasting authorities should resent the replacement of four by sixty-four channels of video signals. What they do not yet appreciate is that their present audiences are not disloyal but merely dissatisfied; they would like more choice, even if in the end they choose to watch what they have always watched, or nothing at all. If the market will bear that, is there any reason why the customers should be denied?

US Public Health Service faces axe

Schweiker urges rethink on OMB cuts

Washington

The Office of Management and Budget (OMB) is proposing broad cuts in health-related programmes for the budget for fiscal year 1984. The National Institutes of Health (NIH) and virtually every public health agency would be affected.

The proposal is contained in OMB's preliminary draft of the President's 1984 budget, which is supposed to be secret until the final budget is published in January. Confidential documents detailing OMB's plans for the health programme, together with a strong protest by Health and Human Services Secretary Richard Schweiker, are now, however, to hand.

According to the documents, OMB wants to limit the NIH budget to \$4,013 million; after inflation, this represents a cut from the current level of \$3,954 million. The short-fall could be made up by "various 'grant-stretching' mechanisms", OMB suggests, such as reducing direct costs of grants by 4 per cent to 20 per cent, cost-sharing with grantees and limiting "excessive personnel compensation". NIH director James Wyngaarden is known to be concerned about the last of these; NIH are at present conducting their own study of the growing proportion of research money used for principal investigators' salaries.

OMB also wants to revive two old and controversial cost-cutting proposals. One is a reduction in the indirect, or overhead, costs that NIH pay universities receiving grants. These payments go for fixed operating costs, such as office space. NIH last spring provoked a storm of protest from the universities when they proposed cutting indirect cost reimbursement by 10 per cent. Congress has since attached language to the NIH appropriations bill forbidding any such cuts. The other proposal is that NIH should charge patients at their clinical centre for routine hospital costs.

Schweiker's appeal against the OMB plan asks for an additional \$229 million for NIH, which would allow the institutes to maintain their present level of 5,000 grants and 10,000 traineeships. The OMB cuts would reduce this to 3,200 grants.

The other Public Health Service agencies appear to fare worse under the OMB plan. The Food and Drug Administration (FDA) would have its budget frozen at the 1983 level and in addition would be required to eliminate 240 full-time positions. The Center for Disease Control (CDC) would have to fire 760 of its 4,162 full-time

employees and reduce its budget by nearly one-third, from \$318 million to \$215 million. The appeal argues that "unless these funds are restored, CDC would lack the capacity to deal with emerging health problems" and would have to "limit research on controlling the 10 leading work-related health problems". It also takes exception to OMB's assumption that FDA could raise \$5 million by charging industry for the cost of processing new drug applications; FDA has not yet determined if "user charges can be collected without compromising the mission of the agency", the appeal says.

Schweiker is asking that OMB should add \$35 million to the FDA budget and allow the agency to maintain its current staff level, and to limit the CDC cuts to \$54 million and 60 full-time positions.

Schweiker is also protesting against organizational changes that OMB included in its budget plan. The most drastic of these would diminish the role of the assistant secretary for health, Dr Edward Brandt, to that of a policy adviser. Brandt, who is considered a powerful advocate of health issues, has operating authority over all of the Public Health Service agencies, including NIH, FDA, CDC and the Alcohol, Drug Abuse and Mental Health Administration; in addition, health promotion and environmental health programmes and the National Centres for Health Statistics and for Health Services Research are run directly from his office. The OMB plan would parcel out these programmes to the various agencies and would eliminate Brandt's authority over the agencies. In effect, the Public Health

Service structure would be abolished; each of the agencies would report directly to Secretary Schweiker.

One White House source said "I think Schweiker will be successful in appealing these massive organizational changes which destroy the Public Health Service". The disposition of OMB and the President towards his other appeals is unknown, although there is strong support for a real increase in NIH funding within the President's Office of Science and Technology Policy.

If OMB's proposed cuts do survive Schweiker's appeal, they still face what is likely to be strong opposition in Congress. "Congress would not be very enthused about any of them", said a staff member of the House Appropriations Committee. The support for health research in Congress was demonstrated earlier this year when Congress added \$312 million to the Administration's \$3,642 million request for NIH.

And a staff member of the House Energy and Commerce Committee, which oversees the Public Health Service, pointed out that the President's budget has tended to carry less weight in recent years owing to the increasing use of continuing resolutions to provide funding. This mechanism is invoked when the new fiscal year arrives and the agency-by-agency appropriations bills have not yet been passed. Continued spending authority is lumped into a single stop-gap bill which a President must sign, or the government ceases operations. This restricts the President's power to impose his version of the budget on Congress.

Stephen Budiansky

Archaeopteryx in hiding

Rumours abound in palaeontological circles in Germany that a new specimen of *Archaeopteryx lithographica* was found by a private collector some time last year. The new find, from the classic Solenhofen-Eichstätt area of Bavaria, is purportedly as good as or better than the two existing complete specimens, one of which is in the British Museum



(Natural History) where it is undergoing a further round of extensive preparation.

Of the five known skeletons of *Archaeopteryx* (the sixth example is a single perfectly preserved feather) the "Maxberg exemplar" (see left) found in 1956 is the least known, as the owner is loth to allow specialists even a glimpse of it and only a preliminary description has ever been published.

Whereas the neighbouring state of Baden-Württemberg has legislation which prohibits the sale of finds of scientific importance without special museum authorization, in Bavaria, where no such legislation exists, it is a case of finders-keepers or sale to the highest bidder. And with prices in the hundreds of thousands of Deutschmarks for Pterosaur specimens, what price an *Archaeopteryx*?

Mike Howgate

Soviet science and technology

Andropov has his say

The Soviet Union will spend 25,500 million rubles on scientific and technological research next year — an increase of 6.2 per cent on 1982. The publication of actual investment figures is somewhat rare in the Soviet media, which prefer to cite increases in percentages only (often referring to inaccessible data-bases). The announcement of this increase — which in real terms represents a rise of some four to five per cent, since inflation in the Soviet Union is running at between one and two per cent annually — was made in a *Pravda* editorial (a traditional Soviet method of announcing a new policy drive) dealing with the speech of Party Secretary Yuri Andropov at last month's plenum of the Central Committee of the Communist Party of the Soviet Union.

According to Mr Andropov, the "great reserves" available to the Soviet Union need to be used "more actively and more consistently". This is basically a reiteration of the Brezhnev policy of modernizing industry by high-priority implementation of research results in industry. This policy, Mr Andropov said, would be continued with even greater impetus — evoking from Academician Anatolii Aleksandrov, president of the Academy of Sciences, the rejoinder that if research results were not being implemented on schedule it was not the scientists but the production ministries which were at fault.

Mr Andropov, however, has some ideas of his own as to how this policy should be pursued. Already the Academy of Sciences carries major responsibility for co-ordinating the research programmes launched to meet the specific needs of Soviet industry and agriculture. Now, it appears, in addition to producing new equipment and "front-ranking work methods", the academy is set to take on a troubleshooting role. Together with the State Committee for Science and Technology and the relevant production ministries, it will be expected to "pinpoint and eliminate" the difficulties hindering technical progress. This approach could be of considerable importance for research planning.

During the past ten years, there has been emphasis on the establishment of local "science centres" (usually created by linking existing institutes in the locality), which were intended to serve the research needs of local industry, frequently on a direct contractual basis. The initiative was normally left to the industrial side, which identified production problems and then sought scientists' help to solve them. The new procedure suggests that scientists will now be expected to identify problems as well as solve them — a procedure which would not necessarily appeal to all enterprise directors. Retooling and redesigning of the production line is a

nightmare for Soviet management, since the regular quarterly, annual and quinquennial targets make no allowances for temporary shutdowns. A zealous scientist urging such a closure in the name of long-term improved efficiency might

Nuclear waste disposal

Synroc presses on in Australia

The Synroc programme — Australia's synthetic rock alternative to glass for the disposal of highly active nuclear waste — is about to move up a few gears with the construction of a cut-price, "commercial-scale" Synroc plant 20 miles south-west of Sydney. The plant, at the Lucas Heights Research Laboratories of the Australian Atomic Energy Commission (AAEC), will be big enough to cope — in principle — with 600–900 tons of spent fuel a year. But in practice it will not see a jot of nuclear waste.

The objective, according to Dr Keith Reeve, leader of the Synroc project at AAEC, is nevertheless to prove that Synroc

well run foul of a director eager to meet his immediate target figures.

Perhaps to overcome such frictions, the recent plenum urged the importance of material incentives for those involved in modernization projects. People who "boldly go for the introduction of new technology", it noted, should not find themselves placed in a "disadvantageous situation".

Vera Rich

classification plant". But AAEC and ANU have only A\$2.7 million to spend, the amount of a government grant for the project, plus staff support from AAEC and ANU. A little over A\$1 million was released in August, with the rest promised over the next two years, for construction in 1983–84 and operation in 1984–85. "I blow hot and cold on whether these dates are optimistic or pessimistic", says Reeve.

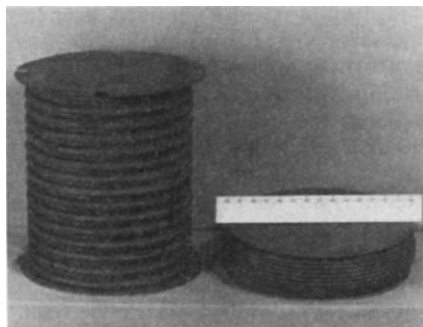
Whenever the plant is ready, it will make Synroc-C, which consists of 60 per cent titanium dioxide, with an admixture of barium oxide, calcium oxide, zirconia and alumina; these react at 1,100–1,170°C and 100–400 atmospheres pressure to create three mineral phases related to perovskite, hollandite and zirconolite. These phases can absorb up to 20 per cent weight of highly active waste oxides, according to Reeves.

At AAEC in Lucas Heights, experiments have concentrated on producing the Synroc in large-scale blocks, rising from a few granules in 1979 to 30-kg hot pressings by the end of 1981. The Synroc itself is able to retain foreign elements much better than glass, the Synroc team maintains; it is also 59 per cent denser and can withstand higher temperatures.

The apparent weakness of the Synroc programme, however, has been the relative lack of work on active Synroc — Synroc actually containing nuclear waste. "We're sensitive to that criticism", says Reeves, and in fact next year Reeves plans a "two-line" radioactive test programme. One will involve neutron-irradiated uranium oxide (to stimulate fission products) in small ($\frac{1}{2}$ –1 cm diameter) Synroc samples, on which leach tests will be done. The oxide will be fresh from irradiation, Reeves stresses, unlike spent fuel which has been cooled for a few years, and it will be possible to reproduce in two years the total beta-ray dose expected in a piece of active, waste-containing Synroc over its lifetime. The samples will then be subjected to leach tests. Alpha irradiation will also be simulated with neutrons.

And in the second line of radioactive tests, Synroc will be made in a glove box with "trace" levels of actinides — neptunium, plutonium, americium and curium. The work will start in June.

Robert Walgate



A recent 30-kg block of Synroc compressed using the uniaxial bellows hot pressing technique. Primary minerals (and waste) are sealed in evacuated stainless steel bellows, and pressed and heated without side supports. The resulting blocks would then be sealed in a larger can.

is a practical alternative to glass. Tenders closed last week for contract management of the detailed design work, and, says Reeve, the successful bidder is likely to have considerable experience of nuclear plant — for AAEC and the Australian National University (ANU), which is also involved in the project, wish to build a plant which, although non-radioactive, is compact, easily maintained, and demonstrates that active Synroc could be made safely.

Cost precluded the construction of a fully active plant, according to Professor Ted Ringwood, inventor of Synroc and ANU representative on the demonstration plant project. A remotely-controlled active plant might have cost "hundreds of millions of dollars" (A\$1.7 = £1), says Ringwood "of the same order as a

West German research and technology

Meeting of past and present

The ceremony last week to open the new London premises of the Anglo-German Foundation for the Study of Industrial Society was attended by the unlikely trio of Dr Heinz Riesenhuber, the present minister at the West German Ministry for Research and Technology (BMFT) and two of his predecessors, Dr Andreas von Bülow and Professor Hans Leussink.

Dr Riesenhuber was in London to discuss Anglo-German cooperation in science and technology with the British government, especially in the context of international organizations such as the European Molecular Biology Laboratory and in information technology. He is also interested in joint industry-government collaboration between Britain and West Germany.

Such work might be managed on the West German side by the federally supported Large Research Centres. Riesenhuber reiterated his aim of directing the work of these centres to more industrial projects and his hope that small to medium-sized firms (fewer than 200

tified before they flower, and with the proposed replacement of student grants by loans (even in secondary education) from 1984 those from modest backgrounds may find it difficult to be recognized. Nevertheless, Dr Riesenhuber believes he knows who the top five in genetic engineering are, and hopes to lure at least one of them to a Large Research Centre.

Dr von Bülow, Riesenhuber's immediate predecessor, was present in his capacity as deputy chairman of the trustees of the Volkswagen Foundation, which has paid

for the renovation of the premises taken over from the Pharmaceutical Society. Since leaving office in October, Dr von Bülow has made known his belief that one of the two experimental nuclear reactors should be abandoned. (The costs of completing the fast and high-temperature reactors were a persistent problem for him as they still are for his successor.) In von Bülow's view, the price of uranium is such a small factor in the overall cost of existing light-water reactors that neither reactor can become competitive in the next thirty years, whatever the price of other fuels. This is in sharp contrast with the policy of the present government to complete both reactors.

J.S. Dunnett

Polish education

New students' association's aims

Poland's new student union, the Association of Polish Students (ZSP), launched last month, has announced a wide programme of action and campaigning to cover virtually all aspects of the higher education process — "the formulation of demands concerning secondary education", equal opportunities of entrance to university, grants, rest and sports facilities, the patronizing of student artistic activities and the "student scientific movement", rationalization of the job market and a loans system for graduates who have difficulties in obtaining housing.

Just how ZSP intends to pursue these aims is not clear — under the new Higher Education Act, which came into force on 1 September, student associations do not have trade union status, and the right to strike is denied them. Some innovations — such as student grants, means-tested on the income of the whole family — have already been introduced by the new act. Other problems, including the disparity of secondary educational standards between urban and rural schools, are already matters of serious government concern. One of the "most important goals" of the association, "exerting influence on matters concerning further education" and the creation of "self-management and democracy" within universities and colleges, is, the statute admits, already covered by the new law, and the association's role will be to lobby for improvements and amendments.

ZSP was founded as a continuation of the old Socialist Association of Polish Students (SZSP), which lost most of its members in autumn 1980, when the Solidarity-type Independent Students Association (NZS) was founded, while many of its functions, including travel and welfare matters, were taken over by "self-government committees" on which both unions were represented. After NZS was banned last January, its members showed no sign of returning to SZSP. Accordingly, shortly before the SZSP Congress (19–20 November), the governing council of SZSP

recommended that the word "Socialist" should be dropped, and that the association should be reshaped to draw in as wide a membership as possible. At the same time, the governing council made clear, the experience of the past ten years should be incorporated and a firm commitment made to socialism. Thus the new ZSP statutes not only acknowledge the "leading role" of the party but also pledge the association to fight for the implementation of socialist values in Poland, and to co-operate with "progressive" youth organizations abroad in the "battle for peace and national and social liberation".

The Congress of SZSP/ZSP met against a background of continuing university unrest. A few days earlier, all teaching in Torun University had been suspended for a week after disturbances in one of the halls of residence. At Warsaw University, the department of psychology was closed for three weeks pending an inquiry by a special "Rector's Commission" into the stoppage of lectures and classes on 10 November, the Solidarity "day of action". (Few universities came out fully in support of this "day", although token stoppages and meetings were reported in several universities, including Krakow and Wroclaw; in Warsaw, there was a rally on the main campus of the university, estimated by the party newspaper *Trybuna Ludu* at around 1,200 persons.) The Warsaw psychology department was allowed to reopen last week following the resignation of the dean and sub-dean, although many students still face disciplinary proceedings.

How far the new association will go to help defuse the situation remains unclear. One innovation proposed for the new ZSP seems to have been designed to provide a ballast of older members, who could well act as a calming influence. In addition to the ordinary student members, ZSP is to admit "supporting members" — Polish citizens who support the statute and who take some active part in the work of the association.

Vera Rich



Heinz Riesenhuber... advocate of collaboration

employees) will band together to support research at the centres. This may simply mean that these centres impinge on the ground at present occupied by the Fraunhofer Gesellschaft, which supports a number of cooperative research laboratories, and independent contract research centres without increasing the amount or effectiveness of research and development. It remains to be seen, however, what form the as yet undefined "indirect" support of industrial research will take.

Last week the minister acknowledged the achievements of West German science under his predecessors and, conscious that research and development is a long-term activity, accepted that change must come slowly. He is, however, concerned about the mediocrity of much basic research and wants to give more support to outstanding scientists. In a constant budget for basic research, this means that money will be less evenly spread. On the other hand, it is not clear how budding geniuses are to be iden-

Stanford graduate students

Clampdown on external links

Washington

Members of the Stanford University faculty will not be allowed to employ their graduate students in jobs in outside companies under a new policy adopted last week by one faculty.

"We felt that this was an area that could be abused", said Dr Gerald Lieberman, dean of graduate studies and research. The concern is that a faculty member may in effect make employment in his outside firm a condition for taking on a graduate student. The new rule forbids employment by a faculty adviser or by a member of a student's dissertation committee. The Massachusetts Institute of Technology has a similar rule.

William Smith, a member of the Graduate Student Association's academic affairs committee, said that he knew of one case where such employment arrangements may have been abused. A graduate student not involved in his research adviser's outside company filed a complaint with the university ombudsman that a fellow student, who was employed by the company, was receiving preferential treatment from the adviser.

The effect of the new rule is unclear. Neither Smith nor Lieberman could say how many students throughout the university are employed by their faculty advisers in outside ventures, although Smith said he knew personally of "about ten" students in that position. But he also said that in any case the rule "won't change anything", because of a loophole that allows exemptions to be granted by department chairmen, so that in effect the rule will be little more than a reporting requirement.

Lieberman, though, said "it puts the burden of proof on the faculty member — I think it's a psychological barrier".

A more controversial proposal will be taken up by the faculty's committee on research this winter; this proposal — recommended by the graduate student association — would require annual reporting by faculty members of all outside activities. A similar requirement was recently proposed by a faculty committee at the University of California at Davis, stemming in part from the controversy surrounding Professor Ray Valentine's involvement with his own company and Allied Chemical that surfaced last spring (see *Nature* 4 March, p.6).

Many members of the Stanford faculty reportedly believe such a requirement to be an intrusion of privacy; Lieberman noted that "the big disadvantage is the feeling that once a faculty member has reported, he's absolved of 'guilt'. That means that if you have reported, someone has to take it seriously", which means reviewing the reports and following up on them where necessary. He questioned whether that

would be done.

In the recommendation from the graduate student association, Smith writes that at least ten undergraduates, graduates and postdoctoral students have lodged

UK nuclear power

Hold on fast reactors

The British government's decision to put its fast reactor programme on ice, made public last week in the House of Commons, has spread consternation throughout the UK Atomic Energy Authority. Although the research establishment at Dounreay in the north of Scotland is not likely to be much affected by the decision, other research establishments at Risley (Lancashire), Harwell (Oxfordshire) and Winfrith (Dorset) are likely to suffer reduced budgets in the years ahead. So too is the team of design engineers maintained by the National Nuclear Corporation on the Risley site, which has been putting the finishing touches to the design of a full-scale fast reactor for the past several years.

According to Mr Nigel Lawson, the energy secretary in the British government, it has been decided that the electricity generating industry will not on economic grounds need to order fast reactors before the early years of the next century, so that the programme of fast reactor development based at Dounreay can prudently be stretched out.

The Atomic Energy Authority has therefore been asked to put forward a revised development programme, taking account of the new time-scale for the commercial application of fast reactors and of the opportunities for international collaboration on such projects. A spokesman said this week that it was hoped to have a new fast reactor strategy by the early spring of 1983.

By all accounts, it has been decided to keep the Dounreay reactor working, chiefly so as to try out novel kinds of uranium and plutonium fuel and to test the efficacy of the pilot-scale reprocessing plant in operation there. The extent to which the Dounreay establishment will become the focal point for research and development on fast reactors will depend on the readiness of others among the authority's establishments to give up their interests in the subject.

In the present programme of research and development by the Atomic Energy Authority, fast reactor work accounts for an annual expenditure of about £100 million. Of this sum, roughly a half is spent in the north of Scotland. Part of Mr Lawson's calculation seems to be that if the authority is told clearly that the programme must be stretched out, the result may be more diligence in looking for

complaints about conflicts of interest on the part of their advisers that they believed interfered with their education and training. The problem, Smith said, centres in the departments of biochemistry, chemical engineering, computer science, electrical engineering, mechanical engineering and medical microbiology.

Stephen Budiansky

partners elsewhere. It seems also however to be understood by those concerned that the National Nuclear Corporation should have a say in the final decision on whatever development strategy is eventually agreed.

The outcome of that process is likely to be uncomfortable for the authority's research establishments. The chances are that, in the spring, the government will be looking to the authority for savings of about a quarter of its current expenditure on fast reactor development. (It is unlikely that even the present government will follow the opinion of some officials of the National Nuclear Corporation that the Atomic Energy Authority's own future should now be reconsidered.) The best solution would be a deal with some comparable authority elsewhere that would postpone the evil day until fast reactors are clearly economic. ●

Dutch scientific research

Good and bad

Waalre, The Netherlands

The Netherlands' two major scientific research organizations have experienced mixed fortunes during the past week. While the Netherlands Organization for the Advancement of Pure Science (ZWO) was finalizing plans to become the first foreign partner to share the British Synchrotron Radiation Source at Daresbury, on the home front the Dutch national applied science organization, TNO, was announcing 550 job losses as a result of a declining government subsidy and reduced orders from government ministries.

On 2 December Professor W.F. de Gaay Fortman, chairman of ZWO, and Professor John Kingman, chairman of the UK Science and Engineering Research Council (SERC) signed an agreement which means Dutch scientists will have access to the Synchrotron Radiation Source at SERC's Daresbury Laboratory. In addition the Dutch partners are to construct a new beamline at Daresbury in 1983–84, fitted with experimental stations to carry out extended X-ray absorption fine structure (EXAFS) and small-angle diffraction (SAD) studies (see diagram). The Dutch share of construction costs will be around £1 million, in exchange for which the British will provide local support for visiting Dutch scientists as well as the

experimental facilities.

The collaboration on the Daresbury facility is the first fruit of an "umbrella agreement" signed between ZWO and SERC in June of this year to promote scientific collaboration between the two countries. Previously a protocol on collaboration in astronomical research was signed in La Palma in June 1981 and the two countries are also cooperating, together with the United States, on the Infrared Astronomical Satellite (IRAS)

future. Kingman is also optimistic that further cooperation with the Netherlands will follow within the framework of the recent mutual statement of scientific collaboration.

The industrial sector too is the one small sign of hope apparent in the recent announcement from TNO. Despite an increase in orders from industry, government cutbacks have resulted in an increase in TNO's annual deficit from 7 million guilders in 1981 to an expected 15



An aerial view of Daresbury Laboratory. The most prominent feature is the 72-metre high NSF tower housing one of the world's highest-voltage Van de Graaff accelerators.

due to be launched on 27 January 1983.

The number of experiments being performed at Daresbury will double during the next few months. The deal with ZWO is one source of the increased work load, but cooperation between SERC and industrial concerns is also on the increase. A consortium of Shell, ICI and British Petroleum is already working with SERC and Professor Kingman hopes that "more money but also more work" will be forthcoming from industry in the near

million guilders in 1983. TNO is to lay off 350 workers in the next four years and a further 200 vacancies will be left unfilled — this represents a cut of around 10 per cent of the staff of 5,000. Research priorities are to be adjusted in response to the crisis, with less expenditure on nuclear energy but more on energy conservation, less materials research and better integration and reorientation of environmental and health research, all politically welcome goals.

Casper Schuur

Satellite broadcasting

Digital Britain

The British Government, not often distinguished by its openness, seems nevertheless to be ready to tell more than the whole truth about the technology of its plans for the eagerly awaited communications revolution. Last week, the Department of Industry issued a formal recommendation by a technical committee of the standards that should be adopted in the design of direct-broadcasting satellites. The first of these is expected to be in service in the United Kingdom in 1986.

As if to emphasize that the traditional technology of the communications industry has been superseded, the report, which runs to 54 pages, is on sale at Her Majesty's Stationery Office (Cmnd 8751) at a price of £5.20 — almost £0.10 a page.

Technically, the recommendation of the advisory panel (chairman, Sir Anthony Part) is however surprising by its daring. The committee has rejected the technical proposals of the British Broadcasting Corporation (BBC), which will operate the first two of the five direct broadcasting channels available to the United Kingdom.

The BBC, in evidence to the committee, had argued for an extension of present analogue codes for specifying the intensity and colour of picture elements on a television screen. In effect, the BBC's proposal would have been a more sophisticated version of the widely-used PAL system in which the transmission of colour information is confined to a small part of the available band width. The basic PAL patent expires in 1983.

In the event, the committee has plumped for a digital system for transmitting television signals. The system concerned, although devised by the Independent Broadcasting Authority, has been offered free of charge to the BBC. What seems to have swayed the advisory committee is the technical superiority of the new system (known as MAC) and the chance that it might subsume both PAL and SECAM, the two broadcasting standards at present used in Europe.

Royal Society

Break ground

This year's annual report from the Royal Society of London, most notably subversive because of what it says on relations between British and Soviet scientists (see *Nature* 3 December), also describes how the society has polled its fellows in the hope of finding out how their universities are surviving and how it is moving (gingerly it seems) towards an assessment of the consequences of nuclear war. The Royal Society, it seems, is on the move, although its direction is as yet by no means clear.

The society's annual report explains that

a questionnaire was sent out in June to all members of the society, asking for comments on conditions in their subjects — not merely in their universities. The general response seems to have been a complaint by established academics that it is no longer possible, in the British university system, to appoint promising young people to permanent or even temporary posts. Replies to this questionnaire seem to have been influential in persuading the Royal Society to urge on the British government the need to recruit more young people to academic posts.

The society has also broken new ground by its decision to contribute to public discussion of the consequences of nuclear warfare. The annual report says that the

annual meeting in November 1981 was partly given over to a discussion of this topic, but that the society's council has not yet decided whether to mount a formal study of the question. The significance of that development is merely that it would, if carried through, be entirely without precedent.

For the rest, the Royal Society has a disappointing tale to tell of its success in spending funds on exchange visits by scientists. In relationship to Western as well as Eastern Europe, it seems, the willingness of British scientists to travel has declined. A new scheme to enable exchanges with Australasia may nevertheless take up the slack.

UK manufacturing

Cambridge Instruments upturn

The British-based Cambridge Instrument Co. Ltd (CIC) appears successfully to have weathered the storm that almost drove it out of business three years ago. The latest half-yearly figures show the company to have made a remarkable return to profitability. The company now hopes to capture a major part of the future market for robotic vision systems and for manufacturing equipment for gallium arsenide semiconductor devices. An average annual loss of £3 million in the three years to 1979 has been converted to a pre-tax profit of £1 million on a £22.7 million turnover in the year ending 31 March, and profits for the first half of the current year are double those for the same period last year.

This dramatic change in the company's fortunes is attributed by people in the company to the management skills brought in by Terence J. Gooding, a "company doctor" from the United States who bought a substantial shareholding in 1979. As the Cambridge Scientific Instrument Company, CIC was in the early 1960s the first to produce a commercially viable scanning electron microscope, and this type of equipment remains the largest part of the business. In the late 1970s, however, the company appeared to come to be resting on its laurels and to have lost sight of some commercial realities; it produced a scanning electron microscope which was far in advance of its competitors in specification but which was also so expensive to build that it could not be sold profitably.

The lessons of that expensive error seem to have sunk in. Now, the company is concentrating on feeding back information about customers' requirements to the development teams and is once again increasing its share of the market for scanning microscopes. As an example of customer-inspired development, the company cites its new large-chamber microscopes, used in the semiconductor industry for the examination of large wafers. Now, a US factory is planned to cope with increased demand.

The company has also recently introduced new products in its range of electron-beam microfabricators. The new system, called "Chiprite", promises to double throughput in integrated circuit manufacture, with very high resolution. Together with what the company calls a "revolutionary" new integrated circuit checking system, CIC hopes by these means to expand its already large share of the market for semiconductor manufacturing equipment.

Robotic vision, on the other hand, seems to have arisen as a spin-off from the existing technology of image analysers. By the simple expedient of fitting these machines with zoom lenses, CIC finds itself able to

apply existing software to the control of industrial production processes.

Another major market that CIC hopes to exploit is that for machinery for the manufacture of large crystals of gallium arsenide. This semiconductor has the substantial advantages over silicon that circuits can be packed more densely, partly because of the lower power dissipated, and that switching times are up to ten times faster.

Gallium arsenide is at present used largely in military applications, but civil applications are likely to flourish in the coming decade, in satellite television links for example. Gallium phosphide, and indium phosphide, two other potentially important semiconductor materials, are also produced on CIC equipment. In this area, the company claims the lion's share even of the coveted Japanese market.

Tim Beardsley

Heavy ion research

France finds niche in GANIL

French heavy-ion physics took a step forward on 19 November when GANIL, the national heavy-ion accelerator at Caen in Normandy, produced its first beam. The accelerator's two principal cyclotrons were coupled and, with the help of a smaller injection cyclotron, accelerated a beam of argon ions to 1.8 GeV (45 MeV per nucleon). The first experiments will begin in January.

The decision to build GANIL (Grand Accélérateur National d'Ions Lourds) was taken in 1975 by the then industry minister, Michel d'Ornano. The project was delayed by an early lack of finance but the now completed facility remains within the initial cost estimates of about FF500 million at current prices. According to the director, M. Claude Detraz, the first experiments should start on 10 January; six months' worth of experiments have been planned.

According to its director, GANIL will carry physicists into an intermediate energy and intensity range of unique interest, making it in some ways complementary to other leading heavy-ion accelerators at Berkeley in California, Dubna (near Moscow) and Darmstadt. The Berkeley Bevalac accelerator is capable of accelerating the heaviest nuclei to energies of 1,800 MeV per nucleon. At such relativistic energies, according to current theories, the 3-quark bags that make up each of the protons and neutrons in the nucleus will, it is hoped, break up, leaving quark matter. At lower but still relativistic energies, collisions between heavy nuclei involve interactions between their constituent nucleons. Models for such collisions are comparatively well developed in West Germany.

Technology drive

Korea

South Korea is to launch an "aggressive drive" to upgrade its technology to international levels. Reporting to a recent technology promotion conference, chaired by President Chon Tu-hwan, the Science and Technology Minister Yi Chong-o described his ministry's new eleven-point plan, which includes the introduction of foreign technology on a "new scale", the promotion of joint research and development projects by Korean institutions and their foreign counterparts and the encouragement of the private sector to set up small and medium-sized technology-intensive corporations in advanced countries. An "active recruitment" programme will be launched by the ministry to encourage South Korean scientists now working abroad to return home and take part in the technology drive.

Vera Rich

The Gesellschaft für Schwerionenforschung (GSI) at Darmstadt in West Germany devotes much of its efforts to lower energy regimes, near the Coulomb barrier at 6–7 MeV per nucleon. In this regime, the nuclei undergoing collisions are left in internal equilibrium. By careful experimental arrangement, the heavy nuclei can be made either to rotate at very high frequency — thus allowing investigation of newly discovered angular momentum states — or to collide gently and form new superheavy elements, the most recent being element 109, which was first synthesized a few months ago. (The accelerator at Dubna is also devoted to such experiments.) Successful models have been established for collisions at these energies that treat the nuclei as "collective" bodies.

GANIL will concentrate on the energy regime between GSI and Dubna, producing sufficient intensity (10^{13} nuclei per second) for rare species such as neutron-rich nuclei far from stability to be investigated (such as ^{20}C). In the next six months, ions of neon, argon and krypton will be accelerated at all energies (the highest for Ar, for example, being 80 MeV per nucleon). Preliminary experiments at CERN in Geneva have given some idea of what may be expected. For instance, there seems to be a limit to the amount of linear momentum that can be transferred to the target nucleus as the beam energy is increased. The reasons for this phenomenon are not known. Such glimpses apart, however, GANIL will be taking nuclear physicists into unknown territory.

Philip Campbell

Intermediate vector boson

No Christmas boon for CERN

The race to discover the intermediate vector bosons, the set of particles supposed to play the role of the photon in the weak nuclear interaction, is in full tilt this week, at the European Centre for Nuclear Research (CERN) near Geneva.

Experiment UA1, the larger of two experiments (a collaboration of 13 institutions involving 128 physicists), already has one or two candidates for the charged boson (the W), but the group cannot be certain yet whether these events are signal or background. The smaller experiment, UA2 (6 institutions and 51 physicists), is being more cautious, and wants time to consider its "public statement".

"Ho, Ho, Ho! You didn't think I'd forget?"



So it seems that unless phenomenal luck strikes the groups this week, and they observe a clear signal for a Z, the neutral boson which is easier to distinguish from background but ten times rarer in the experiment than a W, Christmas may come without the present that CERN director Professor Herwig Schopper was hoping for (see *Nature* 18 November, p.205).

There are two technical problems: low average luminosity and high background. The luminosity is a measure of the frequency of events — collisions between protons and antiprotons producing showers of particles, among which the bosons must be sought. When UA1 was designed, the expected luminosity was $10^{30} \text{ cm}^{-2} \text{ s}^{-1}$, but the current figure is nearer $4 \times 10^{28} \text{ cm}^{-2} \text{ s}^{-1}$, 25 times lower. Thus in the total run-time this year, UA1 expects to have collected only about five Ws but to have only a 50 per cent chance of having collected a Z, needles in a giant haystack of the other particles produced in collisions at the collider centre-of-mass energy of 540 GeV (that is, approaching 600 proton masses of available energy).

Even with the strategy being followed at CERN for detecting the intermediate vector bosons, this background is uncomfortably high. The trick is to look for electrons with energy of 40 GeV emerging perpendicular to the direction of the collision.

The argument is as follows. If a boson,

WNZ, of mass about 80 GeV were formed at rest in the centre of mass, it would decay into two leptons — electrons, neutrinos, or electron and neutrino — with roughly half the 90-GeV decay energy in each particle. These particles could emerge in all directions, whereas the predominant quark-antiquark interactions in collisions between a proton and an antiproton produce only glancing blows and hence little sideways movement.

The objective is thus to detect the sideways 40-GeV electrons. However, gluon-gluon collisions between the "colour" fields of the proton and antiproton can also produce tracks in the detectors which mimic such electrons, and the trick is to distinguish one from the other. In the case of the Ws, which produce only one electron (and one neutrino), this can be done only by distinguishing the average kinematics of the tracks. And of course, to make distinguishable averages, reasonably high numbers of events are necessary. The rarer neutral Z, however, decays into an electron and a positron, both of which should be detected, so making identification much easier.

Next year the search is to be stepped up, with a projected luminosity of $10^{29} \text{ cm}^{-2} \text{ s}^{-1}$ and a three-month run — a plan causing some consternation among other users of the Super Proton Synchrotron (SPS), which was originally intended to fire high-energy protons at fixed targets. When the collider was proposed, there was a proviso that it would never take more than a third of total SPS run-time. Next year the ceiling is to be broken in an effort to get the intermediate vector boson: 43 per cent of experimental time is to go to the collider.

Robert Walgate

Franco-Indian deal

France and India reached a compromise on the supply of enriched uranium for India's Tarapur reactor near Bombay on 27 November — on the eve of a visit to India by the President of France which may result in major economic and military agreements.

Supplies for Tarapur were originally guaranteed by the United States in a 30-year agreement dating from 1963, and backed up by a tripartite inspection arrangement between the International Atomic Energy Agency and the two countries in 1971. But both deals were invalidated by President Carter's Non-Proliferation Act, which forbade the supply of sensitive materials to non-signatories of the international Non-Proliferation Treaty — of which India is one. Carter, who had forgotten — or ignored — the treaties with India, managed to override the act to supply one load of fuel; but President Reagan has felt unable to continue in this manner. France has accordingly stepped into the breach, but began by demanding inspection rights which Mrs Gandhi was unwilling to accept. These guarantees would have gone beyond the terms of the 1963 and 1971 treaties.

In Paris, it was being argued last week that it would have been more dangerous *not* to sign the present compromise agreement than to stand off and let India reject the 1963 and 1971 inspection terms altogether. India already has a considerable amount of waste fuel at Tarapur, which could be reprocessed to extract the plutonium whenever India felt morally free to do so, the justification goes. The present agreement preempts that possibility.

Robert Walgate

Nature index of biotechnology stocks

1982 high	1982 low	Company	Close previous month	Close 26 Nov.	Change
46 1/4	16 1/8	A.B. Fortia (Sweden)	39 1/2	46 1/4*	6 3/4
8	2	Bio Logicals (Canada)	2 1/4	4 1/2	2 1/4
7	3 5/8	Bio-Response (USA)	5	6 1/2	1 1/2
14 1/8	7 1/4	Cetus (USA)	9 3/4	12 1/8	2 7/8
13 1/8	6 1/8	Collaborative Research (USA)	9 3/4	13 1/8*	3 3/8
12 7/8	5 1/4	Damon (USA)	9 1/8	12 7/8*	3 1/2
23 1/2	8 1/4	Enzo-Biochem (USA)	17	23 1/2*	6 1/2
28	6 5/8	Flow General (USA)	13 1/4	13 1/8	-1/8
47 3/4	26	Genentech (USA)	47 3/4	40 1/4	-7 1/2
9 3/4	7	Genex (USA)	9 1/4	9 3/4*	1/2
6 3/4	2 1/4	Genetic Systems (USA)	5 1/8	6 3/4	1 1/8
26 1/4	9 5/8	Hybritech (USA)	20 1/2	25 3/4	5 1/4
14	5	Molecular Genetics (USA)	9 3/4	14*	4 1/4
52	34 7/8	Novo Industri A/S (Den.)	47 1/2	45 5/8	-1 7/8
21	8	Monoclonal Antibodies (USA)	14 3/4	21*	6 1/4

The *Nature* Biotechnology Index for November 1982 stands at 157.4 compared with 140.2 last month. Base is 100 as of 25 June 1982. Previous indexes appeared in *Nature* 12 August, p.599; 9 September, p.101; 14 October, p.573 and 11 November, p.101. Where stocks are traded over the counter, the price quoted is the bid price. For stocks traded on the American Stock Exchange and the New York Stock Exchange, the price quoted is the transaction price. Data from E.F. Hutton, Inc.

* New high or low for this calendar year.

CORRESPONDENCE

Lead in petrol

SIR — Jasper Becker's report (*Nature* 4 November, p.6) on efforts to persuade the European Community to revise its directive on the lead content of petrol perpetuates a general misunderstanding about the directive by saying that it permits a maximum level of 0.4 grammes of lead per litre of petrol and a minimum of 0.15, thus implying that petrol with less than 0.15 grammes is not permitted. Later the report reinforces this misconception by describing the current campaign to introduce lead-free petrol and saying that "no member state is at present allowed to do this".

In fact the directive does not prevent anyone marketing lead-free petrol. What it does do is to prevent any member state from banning lead in petrol or insisting on any lead level less than 0.15 grammes per litre. Thus if the motor industry and the oil companies could agree, it would be possible for lead-free petrol to be introduced tomorrow. If, however, member states are to intervene and insist on lead-free petrol from a certain date they will have to do so simultaneously by agreeing an amending directive.

Another requirement of the directive that deserves to be better known is that the member states have to supply the EEC Commission, at its request, with information on the "development of the concentrations of lead and polluting substances in the urban atmosphere and their effect on public health". The Commission in turn is to report to the Council and Parliament on the information thus obtained and, in the light of the data compiled, is to make appropriate proposals for developing further Community policy on the lead content of petrol. Thus it was foreseen in 1978, when the directive was agreed, that the levels could and should be changed if new knowledge justified it.

NIGEL HAIGH

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No contest?

SIR — *Nature* is highly valued by the scientific community not only for its scientific content, but also for its unwavering position on matters of scientific moral behaviour — and last but not least, the standing of its advertisements. Therefore I would like to draw your attention to an instance which I consider as reprehensible practice, in which, in this case, *Nature* has been unwittingly the vehicle.

In *Nature* Vol. 285, 26 June 1980, the Malaysian government announced (page xxvii) a world-wide competition to find a speedy and accurate method of determining the dry rubber content (DRC) of latex. I sent an entry to this contest on 18 December 1980.

A letter of the Malaysian Rubber Research and Development Board (MRRDB), dated 3 March 1982 stated: "... [we] regret the delay in the final judging; this has been due to the good response [63 entries, note M.B.] received and the need to test out some of the entries".

Without news for several months from the MRRDB, I wrote them a letter on 26 October 1982, from which I quote: "I presume this

final judging has taken place. Sincere scientific curiosity, besides the sportsmanship of having participated, prompts me to ask the communication of the optimal method having been selected. Perhaps it could be published. . ."

From the answer of the MRRDB (10 November 1982): "Judging of the Dry Rubber Content determination competition took place on 28 October 1982 and regretfully, of the 63 entries received from all over the world, including the one entered by you, there has been no outright winner as none of the entries met the criteria of an ideal method for DRC determination."

Conclusion: for the price of an advertisement — and the vague promise of the sum of about \$10,000, which is a mere trifle as research budgets go — the MRRDB did perhaps not obtain the dream-method, but it may be estimated that a significant fraction of the entries yielded enough valuable ideas, elements and propositions to obtain, through very little further expense, the ideal method, provided that one exists. Really, the MRRDB got quite cheaply a great deal of intellectual effort.

Had the advertisement in question appeared anywhere else than in *Nature*, I at least, and perhaps more of the 62 colleagues who sent entries would have perceived beforehand the unilaterality and the arbitrariness behind such a "contest".

MICHEL M. BENARIE

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Vitamin C reviewed

SIR — In reviewing our book "Vitamin C in Health and Disease" (*Nature* 299, 760), Dr Rivers has had to contend with the problem of assessing three books at once and the self-inflicted difficulty of having apparently prejudged the debate on vitamin C. Rivers's view appears to be that the vitamin's only function in man is to prevent the symptoms of overt clinical scurvy. He may be right, but as we emphasize this possibility in the concluding paragraph of our book, it is surprising that he accuses us of having an "atrophied critical faculty". However, he apparently draws this conclusion from a series of comments about the book which are simply not true, and it is these I wish to refute.

He does not exclude our book from his general observation that all three texts claim that vitamin C will not only cure scurvy but colds, cancer and cardiovascular disease. We make no such claim. We include in our evidence on vitamin C and the common cold the statement that randomized, blind, clinical trials have indicated that megadose vitamin C therapy has almost no effect on either the frequency or length of the cold. However, there is some evidence that there are decreases in the severity of the symptoms. For cardiovascular disease and cancer, we suggest that although vitamin C deficiency may be implicated in the aetiology of these conditions, only long-term, large-scale, randomized, controlled trials can confirm or refute such roles for the vitamin.

Rivers makes a more detailed criticism of one section of the book and again he is in

error. In the figure he refers to, there is both a highly significant difference between the two groups and a significant correlation in one of the groups alone, a finding that he was unable to see by eye. To us, this indicates an association between hepatic vitamin C and rate of cholesterol transformation, without suggesting that a cause and effect relationship has been proven. The section which includes this graph makes no claim, as Rivers states, that changes in vitamin C status are central to fluctuation in mortality from ischaemic heart disease. Indeed, we warn that such evidence is only circumstantial and indirect.

In our book on vitamin C in health and disease we have attempted to summarize the available evidence. Dr Rivers may honestly feel that we have leant too far towards those who support a role for vitamin C in the prevention of a whole variety of diseases and he is entitled to his opinion, but he should have supported his views by statements which are correct.

C.J. SCHORAH

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Jungle law

SIR — J. Fleuris (*Nature* 18 November, p.212) is correct in stating that *orang* is Malay for man (though *person* is more accurate, as *orang* has no sense of gender), but *orang utan* has *orang-utan* only as a secondary meaning. Its literal meaning is man (or person) of the jungle (*utan* being the common word for jungle, as opposed to *ulu* which means up-river and has come to mean jungle by extension).

J.R. GOSDEN

Eskbank, Midlothian, UK

Chinese names

SIR — M.C. McKenna (*Nature* 18 November, p.212) oversimplifies when he states that Chinese given names are now hyphenated and the second syllable is not capitalized in English transliteration. While the use of the hyphen to link given names is widespread, it is by no means universal and the use of a capital for the second syllable is at least as common as lower-case.

Moreover it is now common in journals from Mainland China for the given names to be written as one word, for example Chen Yurong. This practice still enables the surname (usually one syllable) to be distinguished easily from the given names (usually two syllables) but the single-syllable given name (such as Pei, in Wang Pei) may still cause difficulty. It is not unknown, either, for "helpful" journals from Chinese and other ideographic language sources to present transliterated names in a Western order, without an obvious indication of their practice.

SUSAN JELIS

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NEWS AND VIEWS

Oncogenes in solid tumours

One of the most obvious quibbles about the recently isolated clutch of human oncogenes should be quenched by the paper on page 539 of this week's issue. Since most of the oncogenes, including the first to be sequenced (see *Nature* 11 November, pp.143 and 149), have been derived from tumour cell lines cultured over many generations, the question has naturally been raised whether the supposed oncogenes might be an artefact of cell culture. But data now reported from the Laboratory of Cellular and Molecular Biology of the National Cancer Institute show that oncogenes can be found in cells taken from a variety of solid tumours surgically removed from human patients some time ago and since kept in a deep freeze. The point is important because it reinforces confidence in the pathological relevance of the discovery reported last month that cells cultured from a human bladder carcinoma contain an oncogene whose structure (described by R.A. Weinberg's group at the Massachusetts Institute of Technology and by Mariano Barbacid's group at the National Cancer Institute) differs from that of a normal human gene by a single substitution of one nucleotide by another. What now also emerges is good (if circumstantial) evidence for at least a second human oncogene. And oncogenes of some kind have turned up in several different types of human carcinoma — the lung (one), the pancreas (one), the colon (two) and an infantile sarcoma known as embryonal rhabdomyosarcoma.

The human oncogenes whose existence has been demonstrated in these solid tumours (and in a number of cell lines derived from a variety of other tumours) are, however, not the same as that reported last month by the Weinberg and Barbacid groups. They showed by sequencing nucleotide bases of an oncogene in a human bladder carcinoma that the oncogene differs from a naturally occurring normal gene only in a single base substitution, the effect of which is to substitute the amino acid valine for glycine in the twelfth position along the length of a protein molecule called p21. (The nondescript name is a sign of how little is known of the normal function of this material: "p" stands for "protein", "21" for the molecular weight, approximately 21,000). On this occasion, the nucleotide sequences of the oncogenes have not been determined directly, but have instead been inferred from the readiness with which cloned stretches of the DNA concerned will combine with the oncogenes of a variety of retroviruses.

The striking result is that most of the human oncogenes now found are related to the oncogenes carried by the retrovirus known as the Kirsten murine sarcoma virus. Indeed, in the malignant human cells, whether from tumours or by cell-culture, the version of the p21 protein produced is often antigenically similar to that produced in mouse cells transformed (or made malignant) by the Kirsten virus. From the known structure of the virus genes, the consequences for p21 should consist of another substitution for the glycine at position twelve. Nobody will be surprised if, in the human cells as in the virus, the glycine is this time replaced by serine, not valine. And given that the retrovirus known as the Harvey murine sarcoma virus is known to produce an altered p21 protein in which the same critical glycine is replaced by arginine, it will no doubt be only a matter of time before some human cancer associated with that mutation turns up. It is also, however, clear that mutations of the p21 gene are not a sufficient explanation of the malignant condition.

One of the intriguing features of this week's report by Barbacid's group is that cells from the two fresh colon carcinomas tested have oncogenes differing both from each other and from

the Kirsten version of the p21 gene (which, nevertheless, turns up in a cell line derived from another colon carcinoma). It will be fascinating to see what are the nucleotide sequences in these genes, as it will be to know why the gene product of the single case of pancreatic carcinoma studied appears to have a molecular weight of 27,000, not 21,000. Meanwhile, Barbacid and his colleagues have taken advantage of the availability of normal as well as cancerous tissue from the patient with the pancreatic carcinoma to demonstrate that DNA from the normal tissues will not convert mouse cells of the NIH line known as 3T3 from their usual (nearly malignant) condition into cells that are frankly malignant. That has an obvious bearing on the question still to be settled of whether human oncogenes of the two types now characterized occur in apparently non-malignant cells, which in turn bears on the question whether in real cells (as distinct from those of the 3T3 cell line) the presence of an oncogene is a sufficient cause of cancer.

Already, however, it is clear that the two human oncogenes now defined are only a small part of this unfolding story. Thus the oncogenes known to be involved in tumours of the white blood cells, leukaemias, lymphomas and the like, but mostly in other than human animals, are quite different. Two important papers on that subject appeared last month in *Proc. natn. Acad. Sci. U.S.A.* (79, 6971 and 6999; 1982), even if their relationship with oncogenes is apparent only in the light of more recent information. Both papers are concerned with plasmacytomas, tumours of B lymphocytes secreting immunoglobulins. In the mouse, it is commonly observed that the end of a chromosome 15 has moved, in malignant lymphocytes, to the region of chromosome 12 where the gene for the heavy chain of immunoglobulin is located. Alternatively, a piece of chromosome 15 may finish up on chromosome 6, where the κ -chain genes are based. Jerry Adams and his colleagues in the Walter and Eliza Hall Institute of Medical Research have now cloned from a plasmacytoma a transcriptionally active segment of chromosome 12 which appears to have been derived from elsewhere, conceivably chromosome 15. And Kathryn Calame (from the University of California School of Medicine at Los Angeles) and her colleagues have identified in two plasmacytomas secreting immunoglobulin A the physical conjunction of a segment of chromosome 15 with part of the gene for the immunoglobulin A constant region gene of chromosomes 12 (to be precise, it is joined to that one of the two chromosomes 12 which is not actively involved in immunoglobulin A production).

From results reported from a recent meeting (see *Nature* 2 December, p.403), it now seems that these two groups — and Marcu *et al.* before them (*Proc. natn. Acad. Sci. U.S.A.* 79, 6622; 1982) — have been observing the translocation of the oncogene known as *c-myc* from chromosome 15 to an active region of chromosome 12 or, alternatively, the transfer of this active region to the site of *c-myc* on chromosome 15. Either way the proposal is that the *c-myc* gene thereby becomes expressed and that this is associated with the oncogenetic process.

As a service to readers, the staff of *Nature* will attempt at intervals in the weeks ahead to draw attention to papers appearing elsewhere which are judged to be important contributions to the quickly developing oncogene story. The assistance of readers and of the authors of papers published elsewhere would be appreciated. Communications (by letter or telephone) should be addressed to Dr Peter Newmark in the London office.

Safe with Cepheids?

from David A. Hanes

MEASUREMENTS of the period of oscillation of the pulsating supergiant Cepheid variable stars have underpinned our estimates of the cosmic distance scale and thus of the age of the Universe for more than half a century. If a study¹ recently published by M.J. Stiff and first brought to the attention of astronomers at the General Assembly of the International Astronomical Union in August is correct, however, this application will have led to serious errors.

By astrophysical good fortune, Cepheids combine several properties which make them potentially excellent indicators of distance: they are intrinsically luminous supergiants, and so can be seen even in external galaxies; their luminosity varies with considerable amplitude, so that they draw attention to themselves in crowded star fields; and they vary with well defined periods which are proportional to their intrinsic luminosities. Thus a simple determination of the period of an individual Cepheid allows the intrinsic luminosity to be calculated — comparison with the observed mean apparent luminosity of the star then yields a direct distance estimate; provided that the period–luminosity (PL) law has been correctly calibrated with reference to Cepheids whose distances are independently known.

The importance of the PL law was recognized shortly after its discovery² in 1912 in a sample of Cepheids at a common distance in the Large Magellanic Cloud, the nearest galaxy outside our own Milky Way. The subsequent identification³ of Cepheid variables in the Andromeda nebula (M31) and the Triangulum nebula (M33) resolved the controversy over the nature of the spiral nebulae, proving that our Galaxy is not unique, and set the stage for the discovery⁴ of the expansion of the Universe. The succeeding half century has seen many revisions^{5,6} of the extragalactic distance scale and inferred age of the Universe, but that scale still depends in large measure on the distances implied by the Cepheid variables (see *News & Views* 299, 297; 1982).

It is in the calibration itself that difficulties arise. Cepheids are young massive stars in short-lived late stages of stellar evolution; thus they are rare. None is close enough to permit a straightforward measurement of distance (say, by parallax triangulation) and the calibration relies largely on the identification of a handful of Cepheids in clusters or associations whose distances can be derived by other often less precise means. Moreover, Cepheids are

found preferentially in the plane of the Galaxy, and may be considerably obscured and reddened by interstellar dust. This problem is exacerbated by the youth of the Cepheids: they may be found still embedded in the locally dust-rich environments from which they formed. Thus it is difficult to assess with what precision we know the absolute luminosities and colours of the calibrating Cepheids.

In external galaxies, further problems arise. There is some evidence⁷ that the PL law differs from galaxy to galaxy, perhaps as a consequence of different heavy-element abundances. Measurements of stellar brightness are difficult in crowded fields near the limits of big telescopes. Perhaps most important, only the extremely luminous Cepheids (those of the longest periods) can be detected in remote galaxies; such stars are not found among the calibrating sample, and extrapolations of the locally calibrated PL law are required. A clear understanding of the law is therefore essential.

What form does it take? It has been argued⁸ that a simple PL law is an incomplete description of the phenomenon. With age, evolving stars change their radial distributions of mass, composition, rate and source of energy generation, temperature and opacity. At certain critical phases, manifested observationally by characteristic intrinsic luminosities and surface colours (or, equivalently, surface temperatures), stars may become unstable to pulsational vibrations. The region of the luminosity–colour plane within which such variations occur is referred to as the ‘instability strip’. Elementary physical considerations suggest the necessity for a three-argument period–luminosity–colour (PLC) law — at a given period Cepheids may exhibit a range of mean luminosities, with the intrinsically brighter at that period being the hotter (bluer) stars, while their fainter counterparts are cooler (redder) — or vice versa. No such relation will arise if the instability strip is very narrow, or if some compensating structural difference fortuitously cancels the dependence on colour. An analysis of available data did, however, originally seem to support the three-parameter PLC formulation — although that analysis⁸ has subsequently been hotly criticized^{9,10}.

Supporters of the full PLC law argue that its use improves the Cepheid distance estimates to the fundamental limit permitted by the measuring uncertainties of the observational data themselves. However, there are difficulties. It is disquieting that the colour coefficient ($\beta = 2.7$) derived^{8,11} for the PLC relation so nearly matches the effect ($\beta \sim 3$) which would be produced by a component of

interstellar obscuration; that is, for Cepheids of given period the fainter (obscured?) stars are redder by almost exactly the amount that would be produced by normal interstellar dust giving rise to the observed dimming. While this may be mere coincidence, it suggests that differential obscuration may be largely responsible for the apparent PLC law, in which case the subsequent technique of determining distance may be erroneous, especially as it depends in certain applications upon lengthy extrapolations of the law.

The recognition of this problem recently sparked two separate critical assessments, by J. Brodie and B. Madore¹² in Cambridge and by V. Clube and J. Dawe¹⁰ in Edinburgh. The Cambridge study was based on Monte Carlo simulations of randomly generated data sets, obeying underlying perfect PLC laws but variously affected by reasonable random errors of photometry and by plausible randomly assigned amounts of obscuration. The conclusion was that the coefficient could be in excess of $\beta = 5$, but that in the presence of moderate amounts of differential obscuration the intrinsic PLC relation is completely masked by the dominant extrinsic trends. On the other hand, the Edinburgh study, which criticized (*inter alia*) the original⁸ semitheoretical determination of the colour term, concluded that near-zero or negative values were favoured.

M. Feast and L. Balona published a swift rebuttal¹³ of the Cambridge study, with the distinction between the analyses being one of mathematical methodology: in the rebuttal, maximum likelihood techniques of arguably greater reliability were shown to reconfirm the original PLC derivation. It is this conclusion that is now under spirited attack by M.J. Stiff¹. Like Brodie and Madore, he uses extensive numerical simulations and introduces all conceivably relevant effects: the photometric measuring errors; the effects of obscuring material, randomly assigned; the presumed true physical width of the instability strip; a spread in Cepheid properties caused by

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mass loss among a sample of originally identical stars; and so forth. His analyses show that the maximum likelihood method systematically overestimates β , whether the data are affected by correlated (obscuration-induced) or uncorrelated errors. Indeed, Stift concludes that it is impossible in such analyses to distinguish between $\beta = 0$ — the irrelevance of a colour term — and $\beta = 2.7$, the generally accepted value: the maximum likelihood estimate is indeterminate. His own analyses merely suggest that β must lie in the broad range 0–3. More importantly, he concludes that the inclusion of the colour term is irrelevant and generally erroneous in the use of Cepheids; he recommends the complete abandonment of the PLC relationship as an indicator of cosmic distance.

Such a conclusion is particularly

damaging for the Sandage–Tammann construction^{6,14} of the universal distance scale, which is already under critical attack^{9,15,16} for other reasons, because their entire scale is predicated on the use of the PLC relationship for Cepheids in nearby galaxies — the first stepping-stones in a hierarchical construction. The present study is more supportive of an alternative treatment¹⁷ of the Cepheid variables by de Vaucouleurs, which, when coupled with other indicators, yields a cosmic distance scale half that proposed by Sandage and Tammann. Thus Stift's provocative results may help (if correct in all particulars) to resolve this outstanding controversy, a subject of often heated and occasionally acrimonious debate for the last decade. Observational cosmologists are eagerly awaiting the next instalment. □

A devescovid slithers along at a rate of about $100 \mu\text{m s}^{-1}$. As large protozoa go — *Paramecium*, for instance — that's not very fast, but since the motors are basically bacterial it isn't a bad speed at all. The protozoan vessel is propelled three or four times as fast as a free-swimming *E. coli* cell, even though its flagella are of similar size and rotate at much the same speed (50–150 Hz). Tamm attributes their celerity to the fact that devescovidins glide over solid substrates — probably bits of wood ingested by the termites — and that less energy is dissipated at a phase interface than free in a fluid.

Can one be sure that it is really these bacteria that are responsible for the motility of the protozoan? Two lines of evidence, at least, indicate that this is so. First, many fragments of protozoan cells — crushed, for example, under a coverslip — rotate actively if they bear bacteria, although they may completely lack any of the four devescovidin cilia: the more bacteria they bear, the faster they spin. Bacteria liberated from crushed host cells can swim, too. Second, a proton ionophore such as dinitrophenol (10 mM), which interferes with the motility of bacterial flagella, reversibly arrests the movement of the devescovidin though not the beating of its own cilia. On the other hand, certain polyene antibiotics, toxic to most eukaryotic cells, inactivate the four cilia of the protozoan but have little effect on its gliding activity. (A specific inhibitor of the ATP mechanism of protozoan cilia would presumably not interfere with the gliding movements of the devescovidins, but Tamm did not mention having tried this.)

How and why could such a system have evolved? We might start by asking what benefit the protozoa could derive from being able to move at all. Perhaps such slithering around enables them to keep making new contacts with the bits of wood on which they are presumed to feed. (Many symbiotic protozoa digest lignocellulose

Microbial galley slaves

from Ralph A. Lewin

SOME of the weirdest and most wonderful microorganisms are to be found in termite guts, veritable zoos of strange protozoa, and among these a devescovidin flagellate is especially notable for having enslaved bacteria to move it around. Some recent papers by Tamm^{1,2} provide revelations on this subject, trumping science fiction with science facts.

Devescovidins are fairly large, as microbes go; their cells are 100–150 μm long and 30 μm wide. They occur in the digestive systems of certain Florida termites (*Cryptotermes cavifrons*), where they slither around in continuous motion. Each cell has a pointed front end tipped with a sort of nose-cone or conning-turret, which is constantly rotating at about 30 r.p.m. (Look at it face-on, and you see that it always turns clockwise. How a single lump of protoplasm can have a swivel like this is one of its mysteries; it must have no microtubules in its 'neck' regions, or they'd soon get screwed up.) At its front end the animal has four whip-like cilia, about as long as the cell and some 400 nm wide, with the usual arrangement of 9 + 2 microtubules found in other eukaryotes. Three of them lash to and fro, forwards and back, while the fourth trails backwards like a sort of rudder. However, the three beating cilia are not the main mechanism of locomotion for this (as yet unnamed) devescovidin, since most of the work is apparently accomplished by some two or three thousand rod-shaped bacteria, 2–3 μm long, attached to its surface. These little galley slaves lie end to end in more than a hundred single files, firmly embedded in

helical grooves. On its outer side each bacterium bears a dozen flagella. These are 4–5 μm long but only 18 nm wide, and are composed of longitudinal rows of flagellin molecules like those of, say, *Escherichia coli* (though in *E. coli* they are borne on all sides of the cell). Some of the flagella from each bacterial cell trail backwards and join those of the cell behind to form a helical bundle about 24 flagella thick. Hydro-mechanical forces are evidently responsible for aligning and synchronizing their movements. As the individual flagella rotate, so do the helical bundles, and this is what propels the protozoan on its way. (Other ectosymbiotic bacteria — longer, thinner and without flagella — are attached along the ridges between the grooves; their role is so far completely unknown.)

Electron micrograph of a rod bacterium (rb) isolated from the surface of a devescovidin. The flagella arise from what would be the exposed surface of the bacterium and are shown in comparison with the doublet microtubules (dmt) from the devescovidin's own flagellum.

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with the aid of hyper-symbiotic intracellular bacteria, but that's another story.) Perhaps motility, with some kind of orientation mechanism located in the conning-tower apparatus at the pointed end, helps to ensure that the devescovinids stay cozily ensconced inside the termite's gut and are not excreted or shed when their insect host casts its skin. Maybe the general stirring of the intestinal contents helps to keep gut fermentations operating effectively. But why don't these protozoa depend entirely on their own ciliary apparatus, and 'go it alone' as do most other motile microbes? Are the three beating cilia too weak to move such relatively large cells around? Apparently so; or perhaps it is cheaper, energetically, to enslave or enlist bacteria to do the rowing than to row oneself. However, as any trireme skipper knows, even slaves have to be fed if they are to continue to man oars effectively. Presumably some nutrients are transferred through the cell membranes of the protozoan grooves to keep the hard-working bacteria going, but so far this subject seems not to have been investigated.

As for the steps by which such a system of microbial enslavement might have evolved, we can only hazard a guess. Many

animals are covered with bacteria; in some invertebrates they seem to be highly specific. Little flagellate cells may shift bigger things around, sometimes fortuitously (witness the rotating eggs of seaweeds such as *Fucus*, spun by the crowds of spermatozooids attracted to their surfaces) and sometimes in an apparently purposeful fashion (as in the green alga *Prasiola*, where the fertilized egg gains the ability to swim by adopting one of the flagella of its successful male partner³). As Tamm reminds us, there is another genus of protozoa related to the devescovinids, *Myxotricha*, found in the gut of another kind of termite, from Australia, which has rows of bracket-like processes along its sides, each bracket bearing a motile spirochaete; by means of these symbiotic cells, it can swim in much the same way as the devescovinid described above, although at only about one-fifth of the speed. This remarkable symbiotic system, in *Myxotricha*, has been known and studied for about 20 years, but Tamm's observations on devescovinids are much more recent and quite as fascinating. □

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Bird coloration and parasites — a task for the future?

from Paul H. Harvey and Linda Partridge

NATURAL selection for defence against parasites may have more significance for evolutionary biology than has often been supposed. Increasing awareness of host-parasite interactions has led to suggestions that they have a key role in population regulation^{1,2}, in the maintenance of genetic polymorphism³, in social organization^{4,5} and even in the evolution of sex⁶⁻⁸. Now, W.D. Hamilton and Marlene Zuk of the University of Michigan have come up with the hypothesis⁹ that parasite resistance is even important in the evolution of bird coloration. The idea is that birds choose mates with bright plumage and complex songs because these characters are correlated with heritable resistance to parasites.

For a character to be heritable genetic variation must exist. Ever since Haldane¹⁰, population geneticists have known that defence against different parasites could lead to the maintenance of such variation in a host species. Most models of the process assume that increased protection against one type of parasite leads to increased susceptibility to another; this has been verified in several cases (see ref. 3), though mainly among plants and their parasites. However, not all models make this assumption. For example, if defence mechanisms

against parasites are costly in terms of potential reproductive success, then when parasites are common genes for defence may be favoured, but when parasites are rare those same genes may be at a disadvantage³.

Genetic variability in the host species can be maintained without there being a single equilibrium point: limit cycles and other patterns of temporally changing gene frequencies are features of some models. If cycles are long, then genes for parasite resistance which are favoured by natural selection in one generation are, on average, those likely to be favoured in the next generation. As a consequence, when cycles are long, genes for choosing mates that have high parasite resistance will be favoured by sexual selection.

But how can animals recognize that potential mates possess the correct genes? One way would be to assess their parasite loads directly: when an animal has few parasites, it means it has high resistance. A more indirect measure might be via plumage characteristics. That, at least, is

the suggestion made in the recent paper by Hamilton and Zuk⁹: "The methods used should have much in common with those of a physician checking eligibility for life insurance . . . the choosing animal should undress the subject, weigh, listen, observe vital capacity, and take blood, urine and faecal samples". Although they cite no relevant evidence, they go on to argue that "general good health and freedom from parasites are often strikingly indicated in plumage and fur, particularly when these are bright rather than dull or cryptic". Within bird species, they predict that the most brightly coloured animals should have low parasite loads, and, *ceteris paribus*, be favoured mates. In contrast, across species, bright coloration should be found associated with high species-specific parasite loads — species subject to a wider variety of parasites will have experienced stronger selection. They were only able to test the latter prediction, although the former is probably the more telling.

Data on four or five haematzoa and on microfilarial worm infestations of passerine birds from five sites were extracted from the literature. Both sexes of 109 different species were scored for degree of 'brightness', and in those same species the songs of the males were scored for 'complexity'. Male brightness (1), female brightness (2), male song (3), male showiness (1 + 3) and bisexual showiness (1 + 2 + 3) show weak but highly significant increases with recorded parasite load across species. The one exception is negative associations of brightness, song and showiness with the generic 'disease' *Toxoplasma*. A footnote to the paper points out that, although these same general patterns of increased brightness, song and showiness with parasite load are found in grouse, the opposite correlations hold for owls.

Hamilton and Zuk conclude that the results conform to their model, although "evaluation of the many plausible and overlapping theories about display and sexual selection, including our new one, is a daunting task and must be left to the future". That is true, but it is unfortunate that their report was not presented at a greater length. Then they could have presented more than summary statistics of their data and they could have considered other correlates of bright coloration among bird species. Two were mentioned and considered to be in agreement with their theory: bisexual brightness and sexual dimorphism are lower on islands¹¹ (there are fewer parasites on islands), and larger-bodied species tend to be more brightly coloured¹² (larger birds provide a bigger target for vectors, and can tolerate more insect bites). But what of the other correlates pointed out by Baker and Parker¹³ in their detailed study of coloration in 516 species of Western Palearctic birds? For example, diurnally active birds are more conspicuous than nocturnally active birds, gregarious birds are more conspicuous than solitary birds and concealed nesters

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are more conspicuous than exposed nesters. Baker and Parker conclude that "in every case the more colourful category is likely to be a less profitable prey to a predator than the more cryptic category" and "that it is possible to explain variation in the coloration of adult birds largely in terms of the selective pressures generated through predation risk". We can also make *post hoc* explanations for why each of those same categories should be subject to higher parasite infestations.

The time has come to make predictions which will enable us to distinguish between these and alternative hypotheses. Surely, after taking possible taxonomic effects into account, different hypotheses should make different predictions about other

ecological, behavioural or morphological correlates of bright coloration? □

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Vaccination programmes and herd immunity

from Robert M. May

VIEWED from the level of the population as a whole, immunization programmes have two primary effects.

The more obvious, or 'direct', effect of vaccinating some fraction of a population against a particular infection is that most of the vaccinated individuals are thereby protected. The ideal vaccine or toxoid is one which produces immunity in all treated individuals (100 per cent efficacy), and where this immunity is lasting. Current measles vaccines, for instance, approach this ideal, apparently inducing lifelong immunity with about 95 per cent efficacy.

Less obviously, the immunization of some fraction of the population is likely to have the 'indirect' effect of diminishing the 'force of infection', defined as the probability that an unvaccinated individual will contract the infection within a specified time interval. If fewer people are candidates to run around coughing or sneezing at you, your chances of acquiring infection are likely to be smaller. One consequence of the existence of such indirect effects is that it is not necessary to immunize everyone in order to eradicate an infectious disease; once a sufficiently large fraction of the population has been protected, the associated indirect effects can drive the reproductive rate of the infection — the average number of secondary cases produced by an infected individual — below unity, so that the infection cannot be maintained^{1,2} (except by immigration). It is the intuitive appreciation of these facts that lead many people to decide their best vaccination programme is one where almost everyone else is vaccinated while they are not, so that they are indirectly protected without incurring any of the risks or inconveniences associated with direct protection. Such

dissonance between the interests of the individual and the interests of the group arises, of course, in many areas of evolutionary biology and elsewhere. In what follows, we shall meet some subtle variations on this theme.

Bearing in mind both direct and indirect effects, we may ask what happens to the population as a whole — to 'herd immunity' — as a result of immunizing, say, 50 per cent of the population.

One answer comes from the extensive mathematical literature dealing with the transmission and maintenance of infectious diseases. Essentially all such theoretical work is based on the assumption of 'homogeneous mixing', whereby new infections appear at a rate directly proportional both to the number of infectious individuals and to the number of susceptibles. That is to say, school and family associations, and all other kinds of social groupings, are averaged out; infections result from binary encounters within a homogenized population. Under this assumption, implementing a vaccination programme that is not sufficiently intense to eradicate an infection will lead to a new steady state in which the number of susceptible individuals is exactly the same as in the unvaccinated population³. At first sight, this result may seem surprising; intuition suggests the proportion susceptible should decrease as vaccination coverage increases.

The theoretical result is, however, easily proved. First, let R_0 denote the basic reproductive rate of the infection, the average number of secondary cases

produced when an infected individual is introduced into a wholly susceptible population⁴⁻⁶; R_0 depends on the basic biology of the infectious agent, and on all manner of social and environmental factors that affect transmission. Next, suppose that only a fraction x of the population is susceptible, the other individuals having acquired immunity either by vaccination or by recovery from natural infection. In a homogeneously mixed population, the average number of secondary infections produced by each infectious individual is then $R = R_0 x$. But at equilibrium each infection must produce on average exactly one secondary infection, $R = 1$. Hence, for an infection that is established at some endemic steady state, the fraction susceptible is $1/R_0$. The answer depends only on R_0 , and is independent of the proportion vaccinated.

To make the result a bit more concrete, consider the example of an infection with $R_0 = 10$ (which is very roughly the case for many childhood infections in Western countries). The equilibrium state before any vaccination has 10 per cent susceptible, with essentially all the remaining 90 per cent having acquired immunity following recovery from infection (the typical infection runs a relatively short course, so the remaining fraction that are actually infectious at any one time is very small although, of course, crucial for maintenance of the infection). Suppose now that we move to a new steady state in which 50 per cent of the population are immunized by a vaccine or toxoid. If there were no indirect herd effects of immunization, the remaining 50 per cent of the population would be divided between susceptibility and acquired immunity in the same proportions as in the original population, to give a total of 5 per cent susceptible and 95 per cent immune (50 per cent from the control programme, 45 per cent following natural infection). The existence of indirect effects, however, leads under the assumption of homogeneous mixing to there still being 10 per cent ($1/R_0$) susceptible and 90 per cent immune (50 per cent from the control programme, 40 per cent following natural infection). By the same token, immunization of more than 90 per cent of the population (or, more generally, more than a fraction $1-1/R_0$) leads to eradication of the infection, by virtue of the indirect effects⁷. In the absence of such indirect effects, almost 100 per cent immunization would be required to eradicate infection from a very large population.

Many people have observed that real populations are rarely, if ever, 'homogeneously mixed', with susceptibles and infecteds bouncing around like the molecules of an ideal gas. The assumption may nonetheless give a good description of what happens on the average; as Bartlett⁸ has written: 'even if a multiplicity of detailed causes is operating to produce the observed broad classes of events, it is often

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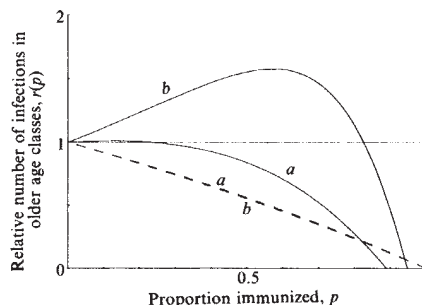
an economy of thought in the sense of Mach to ignore these and appeal merely to the operations of chance and the laws of averages'. This issue cannot be resolved in the abstract. What we need are good empirical tests of the validity of the predictions made by the simple theory.

One especially interesting such test has recently been made for measles by Fine and Clarkson⁹. Analysing the data that are available for age-specific incidence and immunity levels for measles in England and Wales since 1950, they find the 'total number of individuals susceptible to measles has remained relatively constant', at around 4 to 4.5 million. This corresponds to about 9 per cent of the population being susceptible, implying R_0 is around 11–12; this accords with other independent estimates¹⁰. As Fine and Clarkson emphasize, there are biases and deficiencies in the available data (having to do mainly with the notification of cases), and methods of correcting for such biases depend to a degree on the assumption of homogeneous mixing. Thus their test of this assumption is not altogether free of some circularity. I think, however, that Fine and Clarkson's analysis is important, showing that homogeneous mixing represents a useful working approximation for measles in England and Wales, and therefore possibly for broadly similar infections elsewhere.

Weighing risks

In practice, immunization programmes must take account of additional complications arising from age-specific aspects of the vaccination schedules and of the infection processes. If the bad effects of infection are the same at all ages, then any vaccination programme (no matter how low the coverage) will reduce suffering to some extent, provided only that the vaccine is safe. In this situation, it is in principle a relatively simple matter to weigh the health benefits of a vaccination programme against the risks of vaccination itself (although an economic cost-benefit analysis is likely to be more complex, involving the familiar difficulties of assigning monetary values to physical disabilities). The current controversy over vaccination against pertussis (whooping cough) is a case in point. Here the debate is clouded by uncertainties about the risks of vaccination, by the possibility that immunization protects against the symptoms of pertussis but not against becoming infected and infectious (so that no indirect benefits are realized by vaccination) and by a certain amount of emotionalism. I shall not deal further with this vexed subject, lest this article get out of hand.

Such calculations become trickier when a disease has been eradicated from a particular region or country, so that incidence of the disease depends on chance episodes of importation. Becker¹¹ has shown how to estimate the number of fatalities to be expected from such an



The number of infections among females in older age groups (specifically, between 18 and 35 years) when a proportion p of the females are protected by vaccination; the number of infections are calculated as a ratio, $r(p)$, to the corresponding number without any vaccination. The solid curves are for a vaccination policy in which a proportion p of both males and females are immunized at age 2, and the dashed curves are for a policy in which only females are immunized at age 12. The solid and dashed curves a are for $R_0 = 7.5$, and the curves b are for $R_0 = 13$.

episode in an unvaccinated population, before quarantine and vaccination get things under control, and how to weigh this against the number of fatalities to be expected in association with a vaccination programme. This general kind of calculation underlay the UK and US decisions to discontinue routine vaccination against smallpox in 1971: in the UK, the years 1951–1970 saw around 100 deaths from smallpox vaccinations (roughly one death per million primary vaccinations) and approximately 37 from smallpox itself¹.

Age-dependent effects

For many viral infections, the chance of serious complications arising is greater if the infection is acquired at an older age. For example, although the probability of encephalitis occurring as a complication of measles is low, this probability rises roughly linearly with age at infection¹² (at least through the first 20 years of life). In such circumstances, it is important to note that indirect effects of immunization or other factors that diminish the 'force of infection' within a population will correspondingly increase the average age at which infection is acquired. There is evidence to this effect from vaccination programmes against measles and rubella (German measles)^{9,12}.

In short, under a vaccination programme stopping short of eradication, fewer individuals will get sick, but those who do will do so on average at an older age. If fatalities or other complications are more likely at older ages, it is thus conceivable that certain vaccination programmes could actually increase the incidence of serious cases. The likelihood of such a perverse outcome is not easily guessed, but rather requires a close analysis of the detailed interplay among several factors: the way serious illness depends on age, the details of the vaccination programme and the basic biology of the transmission process (summarized by R_0).

It is probable that the history of poliomyelitis represents a 'natural experiment' of this general kind. The argument runs that, in earlier times, most people contracted this infection in the first few years of life, when it very rarely has serious consequences. Increasingly high standards of cleanliness in Western societies in the middle decades of this century led to lower values of R_0 , and thence to a diminished force of infection and a consequent rise in the average age at which infection was acquired (albeit by fewer people, in total). Since paralysis and other complications are apparently much more likely for infections in teenage or older individuals, poliomyelitis paradoxically became more troublesome as its transmissibility declined. More recently, in a notable success story, the advent of safe, effective and cheap vaccines has effectively eradicated poliomyelitis in Western countries.

Rubella is a particularly striking example of an infection whose seriousness depends on the age at infection. Usually a mild infection, rubella can cause the damaging congenital rubella syndrome (CRS) in the offspring of women who acquire the infection in the first trimester of pregnancy. In a seminal paper, Knox¹³ has shown how the incidence of CRS may indeed increase under some vaccination programmes.

The solid curves in the figure (which represents a slightly modified version¹² of Knox's simpler analysis) show the number of individuals who are infected between the ages of 18 and 35 — the main childbearing years in Western countries — when a proportion p of the total population (boys and girls) are successfully immunized at age 2, as a ratio to the number of individuals acquiring infection in this age range before vaccination was introduced. This ratio, $r(p)$, provides a rough estimate of the relative incidence of CRS to be expected for different values of p ; more accurate calculations integrate over the product of the reported age-specific fecundity times the age-specific incidence of infection^{12,13}. Curve a is for an infection with $R_0 = 7.5$ (roughly corresponding to rubella in the US and UK), and curve b is for $R_0 = 13$. In both cases it is assumed that the population is homogeneously mixed, and that everyone lives to age 75 and then obligingly dies (which is a reasonable approximation to the real survivorship function). From these solid curves a and b , we see that the incidence of infection among older individuals can increase under a vaccination programme with moderate levels of coverage (p not too large), and that this propensity becomes more pronounced as R_0 increases.

The dashed curves a and b (which are effectively indistinguishable) correspond to the solid curves a and b respectively, with the one difference that now a proportion p of girls, and only girls, are immunized at age 12. For given p , the two immunization

policies provide the same amount of direct protection against CRS. The 'girls at age 12' policy has little effect on the overall force of infection within the population, and thus produces little in the way of indirect protection; hence the ratio $r(p)$ is roughly just $1-p$. The 'everyone at age 2' policy, on the other hand, does substantially affect the force of infection, producing indirect effects that both increase the average age at infection and eventually, for large p , lead to eradication. Hence, as shown in the figure, the former policy is better at low p and the latter at large p . For rubella, the cross-over point is for p in the range 75–85 per cent^{12–15}.

The solid curve, or 'everyone at age 2' vaccination policy against rubella, is essentially that followed in the US where immunization levels are in the 90 per cent range or above. Conversely, the dashed curve, or 'girls at age 12' vaccination policy, is essentially that adopted in the UK, where p is currently around 70–80 per cent. Each country thus appears to have chosen the correct policy for its particular level of vaccination coverage¹⁶.

This analysis of vaccination strategies against CRS can be elaborated and made more realistic in many ways. In particular, Dietz¹⁴ and Hethcote¹⁵ have included the costs of the different policies in more exhaustive studies.

Most mathematical work on herd immunity and the effects of vaccination concentrates on comparing the initial and final steady states. The dynamics of the transition process, however, can be of great significance, especially as programmes often undergo changes in midstream. Analysis of the age-specific dynamical effects is made complicated by the existence of several characteristic time scales, ranging from around 10 days or so for infection to run its course in an individual, to the 70 years or so that characterize the lifespan and turnover in a population at equilibrium. In some of the

large and avowedly realistic computer models for these dynamical phenomena, apparently harmless approximations (such as updating the force of infection once a year instead of every few days) have led to spurious results because the intricacies associated with the different time scales are not fully understood. I think elaborate numerical studies can only be trusted if

they are grounded on a clear understanding of the basic dynamical processes.

On the other hand, the most mathematically impeccable studies of the dynamics of vaccination programmes are largely fruitless unless tested against factual evidence. There is very little such work, and a clear need for more studies of the kind undertaken by Fine and Clarkson.

Very high-energy gamma-ray astronomy

from P. V. Ramana Murthy and Trevor Weekes

THE high-energy region from 10^{11} to 10^{14} eV is the last window in the electromagnetic spectrum to be exploited by ground-based astronomers and one of particular relevance to high-energy astrophysics. Practitioners in the field met at a recent workshop* in Ootacamund, India to discuss their results: although there have been experiments in four continents over two and a half decades, the techniques used have been somewhat different and there has not always been agreement on the results obtained.

The flux of photons with energies above 10^{11} eV is very small so that detection by satellite, even with larger detectors than at present, would be extremely difficult, if not impossible. At energies greater than 10^{14} eV photons cannot be observed as they undergo photon-photon interactions with the universal 3° black body radiation and are absorbed over distances greater than 10 kiloparsecs. Fortunately for the ground-based astronomers, though, photons in the 10^{11} – 10^{14} eV range are sufficiently energetic that when they interact with the upper atmosphere they produce an electromagnetic cascade that can be detected by its atmospheric Cerenkov radiation using simple light collectors. The great advantage of this technique is that the effective area for detecting γ rays is a few orders of magnitude larger than the geometrical area of the detectors themselves. Although attempts have been made to detect cosmic γ -ray sources using this technique since 1958, it is only in the past decade that sources have been detected.

Cygnus X-3, a binary X-ray source, first came into prominence when it underwent a massive radio outburst in 1972. Within a few days, γ rays of 10^{12} eV were detected from the source. Every year since then the flux, although variable, has been detected (Stepanian, Crimean Astrophysical Observatory). The emission shows two modulations, with 4.8 hour and 34.1 day

periodicity, which also occur at X-ray wavelengths. The 4.8 hour light curve has been seen at energies $\geq 10^{12}$ eV for some time and is quite different from that seen at lower energies; the 34.1 day modulation has only recently been detected (Turver, Durham University) and appears to be out of phase with the X-ray variation.

Since high-energy γ -ray emission has been detected from the young pulsars in Vela and the Crab, it has been suggested that the Cygnus X-3 system may contain a young pulsar. A search for pulsations in the high-energy γ -ray signal in the range 4–40 ms has so far been unsuccessful (Porter, University College, Dublin).

An alternative model based on an analogy with SS433, a source with known jets of relativistic material, requires a black hole or neutron star to be in orbit with a helium star; the rotation axis of the compact object makes an angle with the plane of the system (Grindlay, Harvard-Smithsonian Center for Astrophysics). The analogy is attractive since SS433 is the most powerful source in the Galaxy and Cygnus X-3 may be the most powerful source yet detected.

There is no real problem in producing high-energy γ rays in pulsars; the difficulty arises in getting them away from the pulsar without absorption. Variability in the pulsed γ -ray emission is evidence for this difficulty and suggests that the pulsars that are regular emitters at X-ray and optical wavelengths may be quite different at these extreme energies. Previous reports of transient emission of 15-min duration from the Crab pulsar have been extended by the observation of 1-min variability in the pulsed signal from the Crab pulsar and 15-min variability from the Vela pulsar (Vishwanath, Gupta, Tata Institute of Fundamental Research).

The atmospheric Cerenkov technique finds application in another area — study

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*The 'International Workshop on Very High-Energy Gamma Ray Astronomy' was held at Ootacamund, India on 20–25 September 1982 and was jointly sponsored by the Smithsonian Institution, USA and the Tata Institute of Fundamental Research, Bombay, India.

of extensive air showers produced by ultra-high-energy cosmic rays. By studying air Cerenkov pulse profiles and amplitudes along with other observations, it is becoming possible to understand the charge composition of primary cosmic rays at energies greater than 10^{15} eV (Rao, Tata Institute; Tanahashi, University of Tokyo; Clay, University of Adelaide; Turver).

Further progress in this discipline requires the development of new and more sensitive detection techniques. Improvement in angular resolution will lead to improved minimum flux sensitivities; such improvement may come from the imaging

systems being developed independently at the Crimean and Whipple Observatories. Since the sources so far detected include an X-ray binary, pulsars and a radio galaxy, a small improvement in sensitivity could lead to a dramatic increase in the number of detected sources (Gursky, Naval Research Laboratory). With the development in this decade of more sensitive detectors at satellite γ -ray energies and of new experiments in neutrino astronomy, the role of the atmospheric Cerenkov technique in covering this window in the electromagnetic spectrum will become all the more important. □

supposed postcranial fragments. We agreed that there was no single identifiable fragment of this sample that was human. The remaining fragments were too small for either identification or attribution.

In anthropological terms, the quarrels that this site has provoked have all the makings of a Greek tragedy as harrowing and as long as the *Medea* of Euripides performed so well for the delegates at the open-air theatre at the site. Indeed, the site and the situation now require a cool and informed assessment, perhaps by a commission under the chairmanship of Jan Jelinek, the newly elected President of the European Anthropological Association. Such a commission could consist of an internationally respected small group including a palaeontologist, a palaeolithic archaeologist, a palaeoanthropologist, a cave stratigrapher and a geochronologist. Their task could be to examine the bones, the tools, the cave and the claims made for them.

Make no mistake, however, Aris Poulouanos and his son, Nickos Poulouanos, are to be congratulated on the progress that they have made at the site, but they must also accept that today such a tremendous enterprise must be a team effort. No single researcher can encompass all the disciplines that the rigorous investigation of a site demands today, neither can one man have the time and resources to 'go it alone' successfully.

The view that emerged from the conference was quite clear in that the Petralona specimen is an extremely important skull, almost certainly of Middle Pleistocene age, and of critical importance in documenting the transition from the *Homo erectus* stage of human evolution to the *Homo sapiens* stage in Europe. We owe it to Petralona man, therefore, to gain from the skull and the site every scrap of evidence available to us by the use of modern methods and scientific cooperation. □

Greek fireworks

from M.H. Day

THE Third European Congress of Anthropology was held in Greece at Petralona, near Thessalonika, at the end of September. Two main themes — the pre-*sapiens* stage of human evolution and the biology of migrating populations — attracted great interest and over 200 delegates from around thirty countries took part. One individual dominated the whole gathering, however, and that was Petralona man himself; unfortunately he was not present at the Congress since his fate was then still in the hands of the Greek courts of law. Until the courts decide whether to accede to Aris Poulouanos's request that the skull be transferred to his museum at the Petralona cave, the Petralona skull remains aloof and incarcerated in its present resting place at the University of Thessalonika museum.

The first two days of the meeting were held at the Petralona site itself, in the splendid new facilities that have been provided by the energy and dedication of Poulouanos, including a new tarmac road, a conference hall, a museum and laboratories. The Petralona cave nearby must be one of the most beautiful caves in Europe with hundreds of metres of vaulted galleries with stalactites and stalagmites; the cave is well illuminated and now engineered with walkways. Delegates wandered in and out of the cave freely and were shown anything that they wished to see.

The problems and the scientific disputes over Petralona man are of long standing and now rest principally upon the dating. Indeed, the conference was preceded by a brisk exchange of views on this subject in the pages of this journal¹⁻⁵. The skull itself, billed as 'The Oldest Europaeoid' has features that make it crucial to establish its

evolutionary position.

So muddy have the waters become that almost everything ever stated about the find and the site has been questioned, yet there are some facts that seem to have a solid consensus of support. First, there seems no doubt that at Petralona in 1960 a virtually complete fossil skull was found adherent to the cave wall. Second, the cave is a Pleistocene cave containing an extinct fauna. Third, it does have a stratigraphy that is long and complex. Fourth, there are numerous supposed 'tools' made of bone, limestone, bauxite or quartz. The vast majority of these 'tools' cannot be proved to be artefacts — but some of the quartz tools are undoubtedly humanly worked. Lastly, the cave has clearly been closed for a long time but it is unlikely that any cave would remain closed for 500,000 yr as has been claimed.

This brings us face to face with the problem of dating. Most now agree that the initial estimate of 70,000 yr BP is too young and that on a variety of grounds a date in excess of 200,000 yr BP is more likely. The dating claimed by Poulouanos of over 700,000 yr BP for the skull is very hard to accept on the evidence produced at this meeting. Perhaps the most impressive speakers on dating were M. Ikeya, the originator of ESR dating, and Dr Yokoyama. The consensus of their views would seem to be that 400,000 yr BP would be an appropriate date for Layer 11, the layer said by Poulouanos to correlate with the deposits associated with the skull; but this stratigraphical correlation is difficult to prove over the distance involved in the cave. Various other claims relate to the occurrence of fire at one million years BP, the discovery of the true entrance to the cave used by Petralona man and the recovery of fragments of the Petralona man's postcranial skeleton from the 'Mausoleum' area. Of the latter claim, I can speak personally, having been allowed to examine, with several others, the

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100 years ago

ELABORATE preparations were made in various parts of America to observe the transit of Venus yesterday. The Western Union Telegraph, to facilitate observations, arranged to transmit Washington time wherever desired, in order to secure accuracy in recording results. Some enthusiastic astronomers had proposed general prayer in the churches on Sunday last for clear weather. From *Nature* 27, 132, 7 December 1882.

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Mysteries of the ringed planets

from J. N. Cuzzi

THE colloquium* on planetary rings recently held at Toulouse was designed as a cross-disciplinary meeting of planetary scientists and astrophysicists that would discuss what is known about ring systems and evaluate theoretical understanding of flattened rotating systems in general. The observational material was, not surprisingly, spacecraft-dominated, but the contributions of ground-based observations have been, and will continue to be, of fundamental importance. Theoretical work comes predominantly from gravitational mechanics, and is now moving towards an understanding of collective effects via both fluid-mechanical and statistical-mechanical pathways. Studies of electromagnetic phenomena are also of great importance.

Voyager observations of Saturn's rings and accumulating data from stellar occultations by Uranus's rings provided most of the new observational material. Jupiter's faint ring, first suspected to exist after Pioneer 10 and 11 encounters in 1973 and 1974, was last closely examined by Voyager in 1979. The three known ring systems exhibit certain family resemblances, such as in their proximity to their parent planet and magnetospheric environment; Saturn and Uranus both possess narrow, eccentric rings with abrupt boundaries, and both Saturn and Jupiter possess broad diffuse low-capacity rings composed substantially of short-lived micrometre-sized particles. There are, however, important differences in the composition and sizes of the constituent particles. The rings of Jupiter and Uranus are composed of quite dark, distinctly non-icy material (Nicholson, Cornell University), whereas the bulk of Saturn's much more extensive rings is composed of water-ice. One reported observation of a ring around Neptune remains unconfirmed, there being uncertainty in the position of Neptune's pole (Elliot, MIT). New results on an even larger 'ring' — the asteroid belt — show it to consist of several distinct sub-rings with differing amounts of dry and water-bearing material (Tedesco, JPL).

One fascinating question is that of the relationship of planetary rings to associated 'moonlets', or objects of 1–100 km size. The observed close association and structural influence of such objects, observed for Saturn and inferred for Uranus, have important implications for the origins of the various rings and, perhaps, for the origin of the Solar System as a whole. The Saturn system is generously endowed with moonlets outside the rings. Charged particle data from Pioneer 11

(Van Allen, University of Iowa) and Voyager imaging data (Terrile, JPL) suggest that two of the larger moonlets, the satellites 1980S26 and 1980S27, which apparently confine or 'shepherd' the curiously kinked and stranded F ring, may be only the largest of many smaller moonlets lying in a broad band about 1,000 km wide. It is probably close encounters with some of these moonlets which kink and braid the F ring, but the details of the process are not fully understood either observationally or theoretically (Showalter, Cornell University).

So far no moonlets have been discovered within the main rings, despite careful searches of promising, but limited, regions by Voyager imaging. The presence of large numbers of 1–10 km-sized objects in the rings would clear gaps of comparable width, which seem to have been ruled out by stellar occultation measurements (Esposito, LASP). Furthermore, radio occultation measurements of ring-scattering properties (Marouf, Stanford University) show directly a fairly sharp upper-size cutoff at 5 m, with a broad, possibly power law-size, distribution extending down to about 1 cm. However, the rings may still contain mountain-sized chunks of matter in numbers far less than the upward extension of the power law would indicate. Several have recently been inferred to lie within the 320 km-wide Encke division. This practically empty region is known to contain a few discontinuous clumpy ringlets similar in appearance to the 'kinky' F ring, and gravitational effects by other hypothetical moonlets have been invoked by analogy. New analysis of Voyager images has also revealed azimuthal waves running along the inner and outer edges of the division in orderly patterns which may reflect recent passage by (still unseen) small moonlets (Cuzzin and Scargle, Ames). This is, of course, also indirect evidence but it has a firmer theoretical base. Confirmation of the existence of such moonlets in the rings would help decide between alternative theories of the origin of the rings.

It is becoming increasingly apparent that the large-scale structure of the rings (A, B, C and so on) is highly meaningful, independent of the detailed behaviour of fine internal structure discussed below. In studies of typical length scales from stellar occultation data, Esposito has found no scales preferred over noise with the possible exception of the very largest scales, and has suggested that the significant dynamical processes act on scales far larger than any discussed so far. Combinations of radio opacity (Tyler, Stanford University) and visible-wavelength opacity show that the particle size distribution is significantly different in the A and B rings from that in

the optically thinner C ring and Cassini division; this is in addition to the already known regional differences in particle colour and brightness. Although little attention was given at the meeting to the various origin scenarios, R. Hart (Johns Hopkins University) drew attention to the formative role of the initial energy and momentum of the material, which might also have determined the overall distribution of matter in the planetary system. However, significant dissipative processes may have obscured initial conditions, and ongoing formative processes must be better understood before more detailed inferences as to origin may be drawn.

The question of the fine structure in the rings is yielding to study, but only slightly. The strongest gravitational resonances in the rings are readily identifiable with structure. The two strongest are responsible for the outer edges of the A and B rings, which are radially very sharp (≤ 100 m), and may be comparable in scale with the vertical height or ring thickness. The sharpness of these edges can be understood in terms of local resonant distortion of the particle rotation law from Keplerian, which may allow diffusive transport of angular momentum to vanish (Goldreich, CalTech).

Further detailed calculations (Borderies, CalTech) verify the amplitude of the distortion of the edge of the B ring as due to distortion of resonant streamlines by self-gravity. Weaker resonances produce Lin-Shu spiral waves of either density or vertical displacement at dozens of locations in regions of moderate optical depth, much as predicted by Goldreich and Tremaine several years before Voyager. Almost all the fine regular structure in the outer A ring is probably due to similar, but unresolved, features in excellent positional agreement with predicted resonances with 1980S26 and 1980S27, with the notable exception of the narrow gap near 2.26 Rs, now named Keeler by the IAU. Spiral waves enable the local mass density to be determined and their damping may be used with caution to measure the local viscosity (Lissauer, Berkeley), yielding an estimate of the local vertical scale height of tens of metres in the outer A ring. The question of ring scale height continues to generate controversy; however, a scale height of tens of metres, in a ring composed of centimetre-to-metre-sized particles, would satisfy the 'many-particle-thick' concept favoured by remote-sensing observations such as the opposition brightening (Irvine, University of Massachusetts), radar backscatter (Pettengill, MIT) and radio occultation (Marouf, Stanford University).

In the optically thinner C ring, strong resonances produce very different structure, for reasons not understood. Optically thick ringlets seem to form,

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*'Planetary Rings', IAU Colloquium number 75, was organized by A. Brahic (Meudon) and R. Greenberg (PSI) and held on 30 August–2 September 1982.

usually within or adjacent to fairly clear gaps. Both the ringlets and the gaps show intriguing and unexplained fine structure down to kilometre scale (Lane, JPL). Resonances with Jupiter in the optically even thinner asteroid belt appear to have acted primarily to clear the Kirkwood gaps, but only following the clearing of any massive primordial nebula (Murray, Cornell University). More detailed attempts to assign resonances to the many other features in the C and B rings have been less successful. The enormous number of features calls for comparison with much more numerous, but much weaker, resonances (Wiesel, USAF Institute of Technology). It has been argued alternatively that the uncounted, randomly distributed narrow features in, at least, the B ring may result from a local 'diffusion' or viscous instability, and not from resonances at all. However, early versions of this theory predicted more dramatic fluctuations than observed. Recent refinements have used theoretical understanding gained from dynamical studies of the evolution of particle disks, pioneered by Safronov and recently extended numerically by Brahic, Casanave (CNES) and Clairemidi (Besançon), and analytically by Hornung and Pellat (Ecole Polytechnique). Such results generally indicate different random velocities for particles of different sizes; a sort of 'thermal equilibrium' with the less massive particles being hotter. This refinement appears to smooth the instability-predicted optical depths into better agreement with the observations (Ward and Harris, JPL). However, the problem of the myriad ringlets appears to be a long way from solution.

The narrow eccentric ringlets of Uranus are being explored in increasing detail by occultation measurements in the near-IR where suitable stars are more plentiful and scattered light is limited by methane absorption in the planet's atmosphere. The information has made it possible to determine not only ring eccentricities but also inclinations of comparable amplitude. The eccentricities and inclinations both show a large systematic decrease with increasing distance from the planet, with the exception of the unusually broad and eccentric or outer ring (French, MIT). The observations are, in fact, accurate enough for satellite perturbations of Uranus itself from the system barycentre to be detectable, and direct detection of the influence on the rings of shepherding satellites (Tremaine, MIT), such as, perhaps, distortion of the type seen along the edges of the Encke division, should soon be possible. Certainly the many stellar occultations which will be obtained during Voyager 2 encounter will be extremely illuminating. It is becoming increasingly difficult to make confident assignments of the uranian rings themselves to any three-body resonances once thought appropriate (Aksnes, University of Trondheim;

Tremaine). Postulated shepherding moonlets could, however, be locked in nearby resonant orbits. Because most known narrow ringlets have nearly the maximum eccentricity allowed before orbits start to cross, and because collisions in the rings are common and dissipate angular momentum, postulated shepherding satellites must be nearby to maintain the eccentricity (Tremaine).

In an extensive discussion of gravitational torques and angular momentum transfer, Greenberg stressed the importance of damping processes, which are implicitly assumed in current shepherding formalisms, but which may require special attention in limiting cases of low optical depth or strong interactions. Goldreich also discussed the role of dissipation from the perspective of streamline distortion, and of the tendency of strong perturbations to modify the rotation law sufficiently to cause the normal outward flow of angular momentum to cease or even reverse. Further progress towards understanding the true 'efficiency' of strong resonances and gravitational torques in general may help us to understand at least one puzzle — why the close-in 'ringmoons' are not being hurled away from the rings by their own resonances.

The importance of electromagnetic effects is well established for Saturn's rings, and probably for Jupiter's ring as well. The activity of the 'spokes' in Saturn's rings has been shown to display the periodicity of Saturn's magnetic field, and maximum activity is more specifically associated with the anomalous magnetic longitude on the planet which is, for reasons unknown, also responsible for Saturn's own kilometre-wavelength radiation (Porco, CalTech). Although most spoke features rotate at the local orbital rate, certain sharp nearly radial edges seem to be more nearly co-rotating with the magnetic field (Terrile), perhaps implying ongoing formation. Theoretical work is sketchy but promising, generally focusing on instabilities in current loops running between the rings and the ionosphere (Goldreich), perhaps with subsequent development of propagating clouds of plasma which serve to levitate pre-existing dust grains away from particle surfaces (Morfill, Max-Planck Institut, Garching). Whether the mysterious static, or SED, discovered by the Voyager Planetary Radio Astronomy experiment is connected with the rings (Warwick, Radiophysics, Boulder) or planetary lighting (Burns, Cornell University) remains a subject of lively debate; further observational constraints on power spectrum and periodicity are being obtained (Zarka, Meudon), ground-based detection is being sought (LeCacheux, Paris Observatory) and radio occultation results will eventually be capable of addressing the question of a gap at the proper location identified by one, but not

both, Voyager stellar occultation experiments. Theoretical studies of electromagnetic effects on the behaviour of micrometre and smaller ring particles (Grün, M-P, Heidelberg; Mendis, UCSD) indicate that they have a significant role in the Jupiter ring, perhaps determining the morphology of its 30,000 km-thick 'halo', and probably also in the outer tenuous E, F and G rings of Saturn. The E ring is unlike its neighbours in colour and apparently consists largely of short-lived micrometre-sized particles. This broad ring is centred very close to the orbit of the 250 km-radius moon Enceladus, which itself shows unique surface evidence of recent geological activity, suggesting Enceladus provides the source of ring particles in some way (Johnson, Pang, JPL). The E and G rings are more vertically extended than the main rings, about 1,000 and 100 km respectively (Wassermann, Lowell; Grün, Pedersen), which may be due to electromagnetic effects. However, the problem of the behaviour of charged grains in all of these ring processes is complicated by the stochastic and fluctuating nature of grain charges, both in magnitude (Lafon, Meudon) and in sign (Meyer-Vernet, CNRS).

Many of the processes illustrated in existing ring systems may have been active during the formation of the planets. Safronov (Schmidt Institute) reviewed recent work on nebular evolution. The essence of the problem is transfer of angular momentum as the interstellar medium collapses to form a solar system. The role of viscosity is central; slightly different assumptions as to its source and nature producing qualitatively different results in evolution calculations. Controversy remains as to whether planets formed through such gradual accumulation of planetesimals in a viscous collisional disk or by gravitational instability of nebular gas and dust with subsequent loss of light components. Even electromagnetic effects may have had their day very early in the game. Safronov believes that the very existence of planetary rings, each 'frozen' within its parent planet's Roche zone, attests to the once-ubiquitous presence of a particulate protoplanetary disk.

In summary, although observational and theoretical progress is impressive, there are still many outstanding and intriguing problems. Connections between various ring systems are becoming more apparent. Theoretical studies initiated to understand observed phenomena are aiding basic research into the behaviour of protoplanetary disks. It became clear at the meeting that considerable progress both in theory and observation, could take place in the near future. Further in-depth analysis of Voyager data and a strong continuing programme of ground and orbit-based observations are necessary, and it is hoped that the time will soon again be ripe for further close observation by spacecraft.

REVIEW ARTICLE

Molecular mechanisms of general anaesthesia

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Important constraints on possible molecular mechanisms of general anaesthesia are derived from a quantitative reappraisal of data on the potency of general anaesthetics on whole animals. Despite their popularity, theories that invoke lipids as the prime target do not look at all promising, and available data point much more plausibly to a direct effect on particularly sensitive proteins. Structural changes of proteins on binding general anaesthetics are probably small but may be sufficient to perturb normal function; alternatively, anaesthetics may compete with an endogenous ligand. The phenomenon of pressure reversal of anaesthesia may simply be due to anaesthetics being squeezed away from their target sites.

MODERN surgery would be inconceivable without general anaesthetics. Yet, after more than a century of research, there are very few points of agreement about how and where they act. The extraordinary diversity of theories which have been proposed over the years is matched only by the diversity of molecules which can induce reversible loss of consciousness. Indeed, general anaesthetics form no single chemical class but include substances as dissimilar as xenon, chloroform and thiopentone. This apparent lack of specificity, together with the remarkable observation that general anaesthesia can be reversed by high pressure, poses a unique pharmacological problem.

Unfortunately, rather than converging to a solution, the field at present seems to be diverging along a number of mutually incompatible lines, with various groups becoming entrenched in the defence of particular theories. It therefore seems timely to review the field critically and examine which ideas, in the light of recent data, are really tenable. We hope to show that, despite the present lack of consensus, there are real grounds for optimism.

In this review, we will first discuss what inferences can be made about the molecular nature of the general anaesthetic site(s) from a quantitative consideration of data on the potency of general anaesthetics on whole animals. With this information in mind, we will then assess the relevance of the numerous studies on simple systems and discuss whether lipid or protein provides the most plausible molecular target for general anaesthetic action. (We will not review the literature on isolated nerve preparations, partly because few unifying themes have emerged but mainly because expert and extensive reviews^{1–3} have recently appeared.)

Whole animals

The induction of general anaesthesia must involve a site or a number of sites in the brain. (This is in contrast to local anaesthesia, which results from the blockage of nerve pathways outside the brain.) Because it is not known where in the brain general anaesthetics exert their primary effect, potencies can only be determined using the whole animal. Various behavioural end points, reflecting complex central nervous system activity, have been used and give potencies which usually agree to within a factor of two. Potency is defined as the reciprocal of that concentration (ED_{50}) at which 50% of the animals are anaesthetized, and most quantitative work has rightly focused on the importance of obtaining and analysing accurate ED_{50} values.

Concentrating on anaesthetic end point data effectively means ignoring the large variety of side effects which different general anaesthetics produce and which are of crucial importance to the clinician. The assumption that side effects do not interfere with the anaesthetic end point is, of course, only an approximation; however, a quantitative interpretation of potency data is possible only if this simplifying assumption is made.

What property of the anaesthetic molecule accounts for its potency?

As early as the 1890s, it was known that the potency of an anaesthetic increased roughly in proportion to its partition coefficient (concentration ratio) between olive oil and water^{4–6}. We have shown that an even better correlation is obtained using the slightly more polar solvent octanol, whereas very poor correlations are found with apolar alkane solvents⁷. Although there have been many attempts to correlate potency with various other physical properties of the anaesthetic molecule, none of them has been as complete or successful as the correlations with partition coefficients^{1,8}.

The attraction of these solubility correlations is that they provide a simple physical picture. Since a log-log plot of potency in the aqueous phase versus octanol/water partition coefficient has a unit slope⁷, then, if the general anaesthetic site has solubility properties similar to octanol, all anaesthetic molecules would be equally potent at the site. One must be very careful, when comparing potencies, not to get confused between observed values and the potency at the site itself. For example, in air, ether is roughly five times more potent than cyclopropane⁹, whereas in water it is about ten times less potent⁷; at an octanol-like site, however, both anaesthetics would be equally potent. It is not necessary to postulate that some anaesthetics are intrinsically more effective than others at the site, due, for example, to their relative abilities to break hydrogen bonds¹⁰ or to their relative molecular volumes¹¹. It is also worth pointing out here, while discussing potencies, that a common mistake is to compare^{12–14} potencies in one phase (such as air) with partition coefficients between a solvent and another phase (such as water). The remarkable fact that anaesthetic potency can be predicted from such a simple parameter as the octanol/water partition coefficient not only shows that potency is essentially independent of the detailed size, shape and chemical nature of the anaesthetic molecule, but also strongly suggests that the site is amphiphilic, having both polar and apolar characteristics^{7,15}.

Some drugs could not be considered in the above correlations because their potencies have not been determined. For example, it is very difficult to determine equilibrium potencies for intravenously administered anaesthetics, because of their complicated pharmacokinetics and the fact that (in contrast to inhalational anaesthetics) an effectively infinite reservoir of drug is not present. Other molecules are excluded for potentially more interesting reasons. As one ascends an homologous series, a point is reached beyond which higher members of the series

are not anaesthetic—the so-called ‘cutoff’ effect¹¹. The most obvious explanation for this finding is that the site has a finite size and simply excludes molecules which are too large. While this may reasonably account for decane and tetradecanol being inactive, it is not so immediately obvious why a saturated vapour of C_3F_8 does not anaesthetize mice¹⁶, even after allowing for the much larger volumes of fluorinated compounds¹⁷ compared with their hydrogenated counterparts. What all the ‘cutoff’ molecules do have in common are very low aqueous solubilities. Indeed, it has recently been suggested¹⁸ that because of the low aqueous solubility, it is impossible, in principle, to deliver enough drug to the site, even at equilibrium. However, the final equilibrium concentration at the site must be independent of path; nonetheless, the time taken to reach equilibrium may be so long that, in practice, the drugs appear inactive. We feel that more work is needed before one can say for certain whether or not the observed ‘cutoff’ phenomena reflect fundamental properties of the general anaesthetic site.

How does potency vary with pressure?

Few pharmacologists would expect high pressure to be a particularly useful tool when trying to elucidate the mechanism of action of a drug. However, the intriguing observation^{19,20} that general anaesthesia can be reversed by high pressures (of the order of 150 atmospheres) provides what might be an important clue as to how anaesthetics act. If pressure exerts its effect at the anaesthetic site, then, since pressure can only act by reducing volume, this implies that the total volume of site plus anaesthetic increases when they interact. Although such an increase in volume may be secondary to the anaesthetic mechanism, there are those²¹ who postulate that it is the primary cause. The most self-consistent and best known of these volume expansion theories is the critical volume hypothesis: “Anaesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical amount by the absorption of molecules of an inert substance. If the volume of this hydrophobic region can be restored by changes of temperature or pressure then the anaesthesia will be removed”⁸. According to the theory²¹, as the total pressure is raised, more anaesthetic is needed, simply to counteract the reduction in volume caused by pressure. Figure 1a shows that the quantitative predictions of the critical volume hypothesis are well satisfied using a range of gaseous anaesthetics, for both newts (filled symbols) and mice (open symbols). The slope can only be interpreted if certain unknown properties of the anaesthetic site are assumed; the different slopes for the two animals are said to be due to different site compressibilities and critical volumes^{21,22}.

The impressive agreement between the predictions of the critical volume hypothesis and the pressure reversal data for gaseous anaesthetics has been the main reason for its popular acceptance. However, such agreement is only a necessary but not a sufficient condition for the validity of the hypothesis, and other, more molecularly plausible, theories might fit the data equally well. Perhaps the simplest molecular model is one in which a single anaesthetic molecule binds to the site and inactivates it. This model can be quantitatively tested with the same data used to support the critical volume hypothesis. When this is done (see Fig. 1b), we find, remarkably, that this simple binding model accounts for the pressure reversal data as convincingly as the critical volume hypothesis (see Fig. 1a).

An advantage of this simple binding model over the critical volume hypothesis is that the slopes of the lines in Fig. 1b can be interpreted directly in terms of a single molecular parameter (ΔV), the average partial molar volume of the anaesthetic gases at the site. (Had the potencies been expressed in terms of aqueous concentrations, then ΔV would be the difference between the volumes at the site and in water.) We calculate $\Delta V \approx 190 \text{ cm}^3 \text{ mol}^{-1}$ at the newt site and $\Delta V \approx 65 \text{ cm}^3 \text{ mol}^{-1}$ at the mouse site. These values can be compared to an average partial molar volume of about $65 \text{ cm}^3 \text{ mol}^{-1}$ for these anaesthetics in a simple solvent such as benzene^{21,22}. The larger value for the newt may indicate that, in addition to the expansion

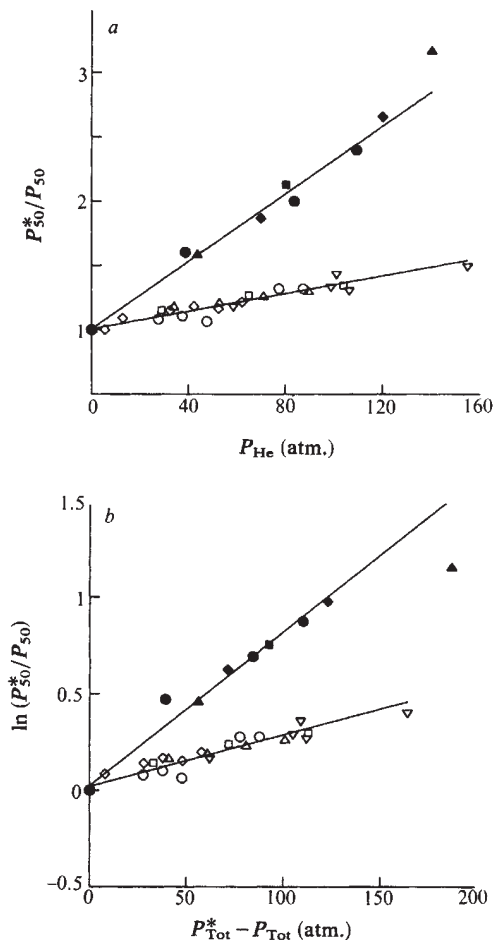


Fig. 1 Pressure reversal data for mice and newts interpreted according to *a*, the critical volume hypothesis²¹ and *b*, a simple binding model. P_{50}^* and P_{50} are the anaesthetic partial pressures needed to anaesthetize 50% of a population of animals at total pressures P_{Tot}^* and P_{Tot} , respectively, and P_{He} is the partial pressure of helium used to increase the total pressure. Open symbols are for the mouse, and filled symbols are for the newt. The anaesthetic gases are: N₂O (○, ●); SF₆ (◇, ◆); Ar (▽); CF₄ (□, ■); and N₂ (△, ▲). The data were taken from refs 21 and 22. In *a*, the data are plotted according to the prediction²¹ of the critical volume hypothesis $P_{50}^*/P_{50} = \text{constant} \cdot P_{He} + 1$, where the constant factor (the slope of the lines) depends upon the compressibility and molar volume of the site, the fractional volume expansion needed to induce anaesthesia, and the partial molar volume and mole fraction solubility of helium at the site. In *b*, the data are plotted according to the prediction of a simple binding model (see text), derived as follows: Let the equilibrium between concentrations of unoccupied site [S], free anaesthetic [A], and inactive complex [SA] be described by the equilibrium constant $K_{eq} = [SA]/([S][A])$. The thermodynamic relationship between K_{eq} and total pressure P_{Tot} is given²³ by $\partial(\ln K_{eq})/\partial P_{Tot} = -\Delta V/(RT)$, where R is the gas constant, T is the absolute temperature and ΔV is the increase in volume of the system when anaesthetic binds to the site. If we assume that anaesthesia occurs when the ratio $[SA]/[S]$ of occupied to unoccupied sites reaches some critical value, then, by integrating the thermodynamic relationship and assuming ideal gas behaviour, we obtain the prediction that $\ln(P_{50}^*/P_{50}) = (\Delta V/RT) \cdot (P_{Tot}^* - P_{Tot})$.

caused by simple solvation of the anaesthetic molecule, there is some additional volume increase due to a small change in the structure of the site itself. (If the site were a protein of weight $100,000 \text{ g mol}^{-1}$ which undergoes a conformational change on binding²⁴, an expansion of only about 0.2% would be sufficient to account for the newt data.) Alternatively, the larger volume could be due to more than one anaesthetic molecule combining with the newt's site. For the mouse, on the other hand, the value of ΔV for these gases is about the same as their average partial molar volume in simple solvents and can thus be accounted for by simple solvation, with little or no additional volume change due to disruption of the site.

It is very often stated that pressure reversal cannot be due to simply squeezing the anaesthetic molecule away from its target site. Our above analysis shows that this simple notion should not be dismissed. Indeed, when one considers the mouse data in Fig. 1b, it is difficult to imagine any site—be it protein or lipid—in the mouse (or presumably in man) for which this would not be the major factor!

The appealing simplicity of the pressure reversal data with gaseous anaesthetics has not been matched by more recent results obtained using nongaseous anaesthetics injected into mice and rats. For these compounds, the decrease in potency with increasing pressure is usually greater²⁵ than that observed with the gases in Fig. 1, can vary significantly from drug to drug²⁶ and can even be nonlinear²⁶. However, we feel that the experimental complications associated with the use of intravenous agents should not be underestimated, and perhaps these results would have been on a surer footing had they been obtained with animals (such as tadpoles) having simpler pharmacokinetics^{25,27}.

Finally, although it is possible to explain most of the pressure-reversal data by assuming that pressure and anaesthetics act at the same site, the possibility that they act at different sites must be seriously considered. For example, pressure reversal could be due to pressure causing a general increase in excitability, which counteracts a general decrease in excitability caused by anaesthetics^{28,29}.

How does potency vary with temperature?

Temperature is a much more familiar experimental variable than pressure, and the effects of temperature on anaesthetic potency have been measured for a number of animals. Nonetheless, the importance of these results has often been underestimated, due, we feel, to the fact that they do not agree with the predictions of most current theories of general anaesthesia.

In 1901, Hans Meyer⁵ found that the aqueous potencies for tadpoles sometimes increased and sometimes decreased with temperature, depending upon the anaesthetic, but that these changes could be largely accounted for by corresponding changes in the oil/water partition coefficients. Later, Kurt Meyer and Hemmi³⁰ reached the same conclusion. More recent work with goldfish¹² showed that potencies expressed in terms of partial pressures decreased markedly with increasing temperature, in line with similar, but somewhat smaller, changes in oil/gas partition coefficients^{31,32}. (It should be stressed that, although oil/water partition coefficients can either increase or decrease with rising temperature, oil/gas coefficients usually decrease³¹.)

In agreement with the goldfish results¹², Eger and his colleagues³²⁻³⁴ found that gas phase potencies for dogs also consistently decreased with increasing temperatures. An obvious problem when working with dogs, or indeed with any warm-blooded animal, is that deviations from the normal body temperature may well produce physiological effects which interfere with the anaesthetic endpoint. At extreme temperatures, this was clearly the case. Over a moderate temperature range, however, this did not appear to be a major complication. Not only were relative changes in potency quite different for different anaesthetics, but for the least soluble anaesthetic (cyclopropane) the change was minimal and could be almost entirely accounted for by changes in solubility. Furthermore,

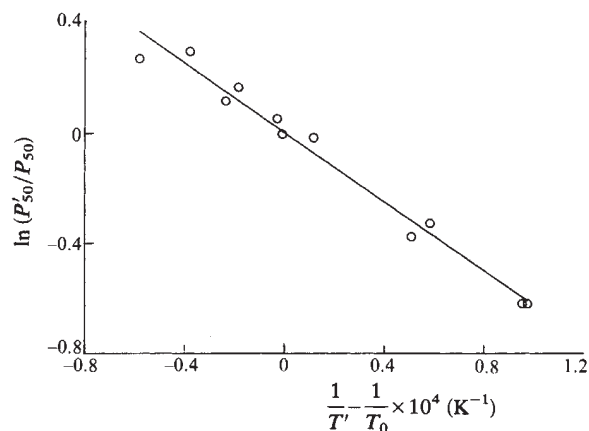


Fig. 2 The effect of temperature on the potency of halothane for dogs, interpreted according to a simple binding model. P'_{50} is the partial pressure of halothane needed to induce anaesthesia at a body temperature T' , and P_{50} is the partial pressure at the normal body temperature $T_0 = 310 \text{ K}$ (37°C). The data³²⁻³⁴ are for dogs with body temperatures T' between about 28 and 43°C . According to a simple binding model, in which a single anaesthetic molecule binds to the site and inactivates it, the equilibrium between concentrations of unoccupied site $[S]$, free anaesthetic $[A]$, and inactive complex $[SA]$ can be described by the equilibrium constant $K_{eq} = [SA]/([S][A])$, which will vary with temperature according to²³ $\partial(\ln K_{eq})/\partial T = \Delta H/(RT^2)$, where ΔH is the enthalpy of binding, that is, $-\Delta H$ represents the heat released when anaesthetic binds to the site. If we assume that anaesthesia occurs when some constant fraction of sites are inactivated, then integration between T' and T_0 yields the prediction that $\ln(P'_{50}/P_{50}) = (\Delta H/R) \cdot (1/T' - 1/T_0)$. For these data, the slope of the line gives $\Delta H = -52 \text{ kJ mol}^{-1}$.

potency changed in the same manner both above and below the normal body temperature (see Fig. 2).

The temperature results pose a serious problem for some popular theories (for example the critical volume hypothesis and lipid bilayer fluidity and phase transition theories), which predict that a rise in temperature should contribute positively to the anaesthetic end point. They can, however, be readily explained in terms of the simple binding model introduced in the previous section. This is shown quantitatively in Fig. 2, where we have plotted the most extensive set of published data (for halothane and dogs). From the slope of the line, we calculate that 52 kJ mol^{-1} of heat is released when halothane binds to the dog site from the gas phase. Heats of binding for various inhalational anaesthetics lie in the range 16 to 56 kJ mol^{-1} for the dog^{32,33} and goldfish¹², with good agreement for a given anaesthetic. The heat released is comparable with, but consistently larger than, that released when binding to olive oil³¹⁻³³ and, although the values for oil are not particularly accurate, the difference is probably real. It might represent some interference with the anaesthetic end point by temperature itself or, more interestingly, it might reflect a genuine difference in binding, indicating that an anaesthetic is more tightly bound at the site than in a simple solvent. Whatever the precise details of the thermodynamics, it is clear that binding models quite naturally account for the consistent experimental observation that gas phase potency decreases with increasing temperature, because of the tendency of heat to drive the anaesthetic off the site into the gas phase.

Molecular models of the anaesthetic site

In a field in which there is so much controversy, it is perhaps comforting to find at least one point of almost universal agreement. Most workers feel that the *ultimate* effects of general anaesthetics are on proteins. The question is: do anaesthetics act directly on proteins, or do they act indirectly by first affecting membrane lipids? An attraction of the idea that membrane lipids may be the primary site is that anaesthetic potencies would be easy to account for³⁵, since both aqueous potencies⁷

and lipid/water partition coefficients³⁶ correlate well with octanol/water partition coefficients.

Lipid bilayer fluidity. Soon after the introduction of magnetic resonance techniques to the study of membranes, it was shown³⁷ that anaesthetics could increase the fluidity of membranes and lipid bilayers. These results led to the popular theory that anaesthetics act by increasing the fluidity of membrane lipids, which, in turn, perturbs the normal functioning of some crucial membrane protein, such as a nerve channel. A large number of subsequent studies, using a variety of techniques, apparently lent support to this idea. In retrospect, however, it is clear that, almost invariably, very high concentrations of anaesthetics were used to obtain the observed increases in fluidity^{7,38}. More recent work^{7,38-41} has shown that, at clinically relevant concentrations, changes in lipid bilayer structure and fluidity are virtually undetectable. The few reports that fluidity is significantly altered at surgical concentrations are either contradictory^{42,43} or else have been obtained at higher than the minimum alveolar concentrations⁴⁴.

Thus purely quantitative considerations argue against changes in bilayer fluidity being important. Nonetheless, it has been argued⁴⁵ that measurements at high concentrations, where clear effects are seen, are relevant and can be extrapolated back to clinical concentrations. This is a risky procedure, since it is extremely difficult to demonstrate linearity of response at low anaesthetic concentrations, where barely measurable effects occur. Interesting information can, however, sometimes be obtained. Such experiments have shown that high concentrations of anaesthetics can sometimes order and sometimes disorder, depending upon the anaesthetic and spin-probe used as well as on the composition of the lipid bilayer^{18,45-47}. While this diversity of observed effects has been used⁴⁶ to argue against fluidizing theories, it has also, on the other hand, encouraged proponents of such theories to 'fine-tune' their systems so that consistent responses are observed with all anaesthetics. Following the latter philosophy, Miller and Pang⁴⁵ concluded that a suitable bilayer must contain at least 10% (molar) cholesterol and have a charge density of between 0.12 and 0.46 charges per phospholipid.

At surgical concentrations, however, fluidity increases in such bilayers (estimated by extrapolation) would be exceedingly small. Indeed, equivalent increases in fluidity occur in cholesterol-containing bilayers which have been warmed only a few tenths of a degree^{41,48}. Needless to say, such small changes in temperature do not induce anaesthesia in whole animals.

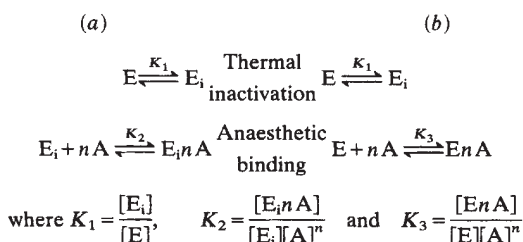


Fig. 3 Two alternative schemes to explain the inhibition of luciferase by inhalational anaesthetics. In both schemes, the active form (E) of the enzyme is in reversible thermal equilibrium with an inactive form (E_i). The schemes differ only in regard to which of these forms the n anaesthetic molecules (A) are assumed to bind. In *a*, binding occurs only to the thermally denatured form¹⁰⁷, while in *b*, binding occurs to the active form of the enzyme. Both schemes fit the data¹⁰⁷ equally well and give reasonable values for the enthalpy increase ($\Delta H_1 = 320 \text{ kJ mol}^{-1}$) and entropy increase ($\Delta S_1 = 1075 \text{ J K}^{-1} \text{ mol}^{-1}$) upon thermal denaturation, and they show that about one anaesthetic molecule ($n \approx 1$) binds to each luciferase molecule. Scheme *a* yields extremely large changes in enthalpy ($\Delta H_2 = -335$ to -376 kJ mol^{-1}) and entropy ($\Delta S_2 = -1,160$ to $-1,340 \text{ J K}^{-1} \text{ mol}^{-1}$) upon binding of the various anaesthetics from the gas phase. It is easy to show that $\Delta H_3 = \Delta H_1 + \Delta H_2$ and $\Delta S_3 = \Delta S_1 + \Delta S_2$, so that scheme *b* yields much smaller and more reasonable values ($\Delta H_3 = -15$ to -56 kJ mol^{-1} and $\Delta S_3 = -85$ to $-265 \text{ J K}^{-1} \text{ mol}^{-1}$), consistent with simple binding.

Moreover, fluidization theories predict an increase in anaesthetic potency with rising temperature (since fluidity increases markedly with temperature), whereas, as we have seen above (Fig. 2), the reverse is observed experimentally. At first sight, one of the attractions of these theories is that pressure might reasonably be expected to counteract the fluidizing effect of anaesthetics, so that pressure reversal seems easy to explain. While qualitatively this prediction is supported by experimental observations, quantitatively the agreement is very poor. The fluidity increases at concentrations which cause general anaesthesia would be counteracted by about 25 atmospheres or less^{39,49,50}, whereas pressures of the order of 150 atmospheres are necessary to overcome anaesthesia in the whole animal. In summary, it seems to us most improbable that changes in lipid bilayer fluidity play any significant role in general anaesthesia. **Phase transitions in lipid bilayers.** Another class of lipid theories supposes that certain bilayers in nerve membranes are poised near a chain-melting phase transition, and that anaesthetics induce a cooperative structural change. Most general anaesthetics do in fact significantly depress the temperature T_m of the gel to liquid crystalline phase transition in pure lipid systems at clinical concentrations⁵¹⁻⁵³. However, the observed effects are equivalent to those produced by a temperature increase of less than a degree⁵¹⁻⁵³ and can be reversed by only a few tens of atmospheres of pressure^{52,53}. Also, some anaesthetics (the higher alcohols) can actually increase^{46,54,55} T_m . Thus these theories suffer from most of the shortcomings of the fluidization theories. An attempt has been made to 'fine-tune' phase transition theories, by introducing lipid mixtures which undergo lateral phase separation⁵⁶. However, not only do difficulties with temperature remain, but two equally potent anaesthetics have been shown¹⁸ to shift solid/fluid phase equilibria in opposite directions.

Finally, it should be remembered that there is little convincing evidence for the existence of significant regions of solid-phase lipids in mammalian membranes. Indeed, in view of the high concentrations of unsaturated hydrocarbon chains in these membranes and the high concentrations of cholesterol in nerve plasma membranes in particular, one would need a large measure of faith to remain convinced that the answer to general anaesthesia lies in the study of lipid phase transitions.

Changes in membrane dimensions. When anaesthetics partition into membranes, some geometrical changes are almost certain to occur. Early workers⁵⁷⁻⁵⁹ found that clinical concentrations of general anaesthetics produce sizeable changes in the surface pressure of lipid monolayers held at a constant area at an air/water interface, and these correspond to smaller but still significant increases in area (of about 0.5%)⁶⁰ at constant pressure. More importantly, very similar increases have been observed in the area of intact red blood cell membranes⁶¹.

The effect of anaesthetics on membrane thickness is much more controversial. Using X-ray and neutron diffraction, we could detect no significant change in the structure or thickness of lipid bilayers exposed to surgical concentrations of nitrous oxide, halothane or cyclopropane^{7,38}. Haydon and his colleagues, on the other hand, showed that high concentrations of the lower alkanes cause significant decreases in the electrical capacitance of black lipid films⁶² and squid axon membranes⁶³, which they reasonably interpreted as thickness increases. Alkanes have also been observed to increase bilayer thickness in multiwalled lipid vesicles⁶⁴. A large increase in thickness (a capacitance decrease) was also reported to occur in solvent-containing black films exposed to benzyl alcohol⁶⁵. However, this report now appears to have been misleading, since later workers⁶⁶⁻⁶⁸ have shown that, when 'solvent-free' films are used, benzyl alcohol, octanol and chloroform actually increase the capacitance. While this might suggest that these agents decrease bilayer thickness, the observed increases in capacitance could be largely due to increases in dielectric constant⁶⁸. Looking at all available results, on fluid lipid bilayers^{7,38,40,66}, 'solvent-free' black films⁶⁶⁻⁶⁸, and myelin membranes⁶⁹, and extrapolating where necessary to low concentrations, we are

forced to conclude that any changes in membrane thickness at general anaesthetic concentrations must be very small.

There has been considerable confusion about the size and importance of the volume changes that occur when anaesthetics enter membranes. On the basis of area expansion and mass density measurements^{61,70,71}, Seeman calculated that while lipid bilayers expand by only the van der Waals volumes of the occupying drug molecules, biological membranes expand by about 20 times this amount. We have recently reinvestigated this problem and find that, in fact, the volume expansion caused by a molecule of halothane is essentially the same in both red cell membranes and lipid bilayers, and little different from that in liquid halothane or water⁷². Kita *et al.*⁷³ have also measured these volumes for a number of different lipid bilayers and anaesthetics and found values close to those in the pure liquid anaesthetics but slightly greater than those in water. In terms of total membrane volume, both red cell membranes⁷² and lipid bilayers^{72,73} expand by only a few tenths of a per cent at clinical concentrations.

In summary, small increases in both area and volume have been detected at clinical concentrations of general anaesthetics, but whether or not changes in thickness occur is uncertain. An estimate of the thickness change can be made, since a small fractional change in volume must equal the sum of the fractional changes in area and thickness. For red cell membranes and halothane, the observed volume expansion⁷² of about 0.1% and area expansion⁶¹ of about 0.4% imply a thickness decrease of about 0.3% (less than 0.2 Å). The possibility of anisotropic changes in membrane dimensions of this sort has been discussed by others^{21,74}. What is important, however, is that changes in membrane dimensions at clinical concentrations are very small indeed. (Comparable changes are produced by only slight increases in temperature: area⁷⁵, thickness⁷⁶ and volume⁷⁷ all change by $\geq 0.1\%$ per degree.) Thus we feel that changes in membrane dimensions do not provide a very plausible basis for theories of general anaesthesia.

Permeability changes in lipid bilayers. Probably the most promising line of argument for those who believe that anaesthetics act primarily on lipids would rest on the effects observed on bilayer permeability. Bangham and his co-workers^{59,78,79} showed that the leakage of cations from lipid vesicles is markedly enhanced by general anaesthetics and that these changes can be reversed by high pressure. At clinical concentrations, these permeability increases can be as large as 20–50% for cations⁸⁰, but, interestingly, appear to be much smaller for anions⁶⁸ and non-electrolytes^{68,79}. Although the permeability of lipid bilayers to inorganic cations is very small, and orders of magnitude less than that observed in biological membranes, similar effects of anaesthetics and pressure have been observed on the much larger cation fluxes found with ionophore-containing bilayers^{78–80}.

While these results are certainly tantalizing, it is not obvious that cation fluxes mediated by ionophores such as valinomycin and gramicidin A are appropriate models for ionic movements across natural membranes. Recent experimental results⁸¹, however, suggest that proton permeabilities across pure lipid bilayers may be anomalously high, and Bangham and Mason⁸² have proposed that anaesthetics act by affecting these proton movements. They suggest that anaesthetics act by collapsing pH gradients across catecholamine-containing synaptic vesicles. This in turn neutralizes the catecholamines (which had been charged in the acidic interior and thus effectively trapped⁸³) and allows them to escape, thereby inhibiting synaptic transmission. Convincing experimental evidence in favour of this new and ingenious theory is still rather sparse, and whether or not lipid bilayers are highly permeable to protons is an extremely controversial point^{84,85}.

Proteins. At first sight, the idea that anaesthetics act directly on protein might seem improbable, since the well constrained structures of most proteins would not be expected to provide the sort of binding environment necessary to account for the simple correlation of anaesthetic potency with octanol solubil-

ity⁷. On the other hand, it might be argued that if the anaesthetic site were an integral membrane protein, its necessarily intimate association with membrane lipids might well provide an octanol-like environment at the protein/lipid interface. However, a major experimental problem which arises when working with integral membrane proteins is that it is difficult to disentangle effects on protein from those on the surrounding lipids. While negative results are meaningful, positive results can be difficult to interpret. To avoid these difficulties, we will focus our attention here on the evidence that has been obtained using simple, relatively pure protein systems, where the observed effects can be interpreted reasonably unambiguously in terms of direct protein/anaesthetic interactions.

Quantitative data on the binding of general anaesthetics to proteins are, unfortunately, not very extensive. However, certain anaesthetic alkanes^{86,87} and alcohols⁸⁷ have been reported to bind to bovine serum albumin (BSA). In addition, Hansch and his colleagues have accumulated a large amount of data on the binding of small organic molecules (including some anaesthetics) to BSA⁸⁸ and haemoglobin⁸⁹ and have shown that the binding of a molecule can be simply correlated with its octanol/water partition coefficient; although not directly relevant to anaesthesia, these data do illustrate that a diverse range of molecules can bind to a protein in a manner relating to their solubilities in a simple organic solvent. Further, using what data^{86–88} are available for the binding of anaesthetic molecules to BSA, we calculate that at concentrations which cause general anaesthesia there would be one or more anaesthetic molecules bound to each protein molecule.

In some cases, organic molecules apparently bind at the interfaces between protein subunits⁸⁹ or subunit-like domains⁹⁰. For example, a number of molecules which bind strongly to haemoglobin do not bind measurably to myoglobin⁸⁹. On the other hand, the small anaesthetics xenon, butane and pentane bind strongly to both haemoglobin and myoglobin⁹¹, apparently in the well-defined pockets which have been identified crystallographically by Schoenborn⁹². β -Lactoglobulin⁹³ and adenylate kinase⁹⁴ are other examples of proteins which fortuitously bind anaesthetics in localized pockets. That such pockets will accept molecules only up to a certain size is illustrated by the alkane-binding site of β -lactoglobulin, which can accept two butane molecules equally well, two pentane molecules unequally well, but only one molecule of iodobutane⁹³. Although all of these binding studies have been performed on proteins which are obviously not directly involved in general anaesthesia, they do illustrate that the solubility properties of the general anaesthetic site, including the 'cutoff' effect, could be quite plausibly mimicked by a protein.

Binding of an anaesthetic to a protein and inhibition of its function are, of course, two very different things. For example, when anaesthetics bind to haemoglobin, the resulting structural changes are very small^{92,95} and the oxygen dissociation curve is unaffected⁹⁶. In contrast to the paucity of data on the binding of anaesthetics to proteins, there is no shortage of reports of direct effects of anaesthetics on protein function. However, if these results are looked at critically, it is clear that in most cases they have little relevance to general anaesthesia: the effects that are or would be observed at clinical concentrations are either very small or undetectable, or else clear effects are seen with some anaesthetics but negligible or opposing effects are seen with others. This is true in most studies with soluble proteins, organized protein assemblies, and membrane proteins^{96–102}.

One class of proteins, however, which do appear to be particularly sensitive to general anaesthetics are the light-emitting luciferase enzymes. Work with whole cells^{103–105} has shown inhibition at low anaesthetic concentrations and even, in some cases, pressure reversal. Although these results are seductive, molecular interpretation is difficult, and there have been too few studies using cell-free systems. A membrane-free preparation of bacterial luciferase has been shown to be inhibited by low concentrations of ether, which apparently competes with

the binding of an aldehyde necessary for luminescence¹⁰⁶. Using a cell-free extract from firefly tails, Ueda and Kamaya¹⁰⁷ showed that firefly luciferase is inhibited by surgical concentrations of halothane, methoxyflurane, chloroform, enflurane and fluroxene. They measured gas-phase potencies at different temperatures but, unfortunately, interpreted their results in terms of a scheme (Fig. 3a) inherited from earlier workers¹⁰³, in which anaesthetics could only interact with a thermally inactivated form of the enzyme. This scheme gave extremely large decreases in enthalpy and entropy on binding, from which they concluded (we think erroneously) that anaesthetics cause a major conformational change when they bind to luciferase.

However, the luciferase data¹⁰⁷ can be interpreted much more reasonably in terms of a simple binding model (Fig. 3b) similar to that introduced in previous sections, in which the anaesthetic binds to the active enzyme and inhibits it. We find that, not only are the enthalpy and entropy changes modest and easy to account for by simple binding (comparable enthalpy changes are observed when these anaesthetics partition from the gas phase into oil³¹⁻³³), but the heats of binding are now similar in magnitude to, and of the same sign as, those found when applying the model to whole animal data. In other words, the temperature dependence of anaesthetic potency in whole animals can easily be accounted for in terms of anaesthetics binding to protein.

Thus, although the data on anaesthetic interactions with proteins are rather sparse compared with those available for lipids, examples of proteins can be found which satisfy most of the properties required of a successful molecular model of the general anaesthetic site.

Conclusions

There are many who would argue that it is premature to search for the molecular mechanisms underlying general anaesthesia. Nonetheless, when one focuses on quantitative data on anaesthetic potencies for whole animals and considers how these potencies vary with pressure and temperature, certain clear trends emerge, which do provide important information on the molecular interactions involved and impose severe constraints

on the molecular nature of the target site. For the relatively inert anaesthetics, for which unambiguous potency data exist, these trends are surprisingly simple. In contrast, complications arise with the intravenous agents. While this may indicate that some anaesthetics act differently from others, it may only reflect the more complex pharmacokinetics of these compounds.

Using the constraints imposed by the whole animal potency data, we have assessed the relevance of simple lipid and protein model systems. Despite the vast literature on anaesthetic/lipid interactions, those theories which postulate that anaesthetics exert their primary effects on lipids do not look at all promising, although changes in lipid bilayer permeability remain a possible candidate. In most cases, the results obtained with proteins look equally unpromising; nevertheless, some proteins do exist which are sensitive to surgical levels of general anaesthetics and are inhibited at anaesthetic concentrations which not only correlate well with whole animal potencies, but also show analogous dependencies upon pressure and temperature. Taken as a whole, the data available point much more plausibly to protein rather than lipid as the primary site of action.

We feel that the simplest working hypothesis, best supported by available data, is that general anaesthetics act by binding directly to a particularly sensitive protein (which may or may not be membrane-bound) and inhibiting its normal function. The fact that potencies correlate with octanol, but not hydrocarbon, partition coefficients suggests that the binding site on the protein is amphiphilic. The anaesthetic could inhibit either by interfering directly with catalytic or transport activity (for example by disrupting subunit-subunit contacts) or by competing for the binding of some endogenous ligand (such as a neurotransmitter). In either case, the structural perturbations which occur on binding are probably small, since the volume and enthalpy changes, derived from the effects of pressure and temperature, are modest. Contrary to popular belief, pressure reversal may well be due to pressure simply 'squeezing' the anaesthetic molecules away from their target sites, while the decrease in gas-phase potency with increasing temperature may similarly be due to heat driving the anaesthetic off these sites.

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ARTICLES

Sr isotopes in interstitial waters of marine sediments from Deep Sea Drilling Project cores

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Sr isotopic compositions of interstitial sea waters have been documented in detail with cores from 37 Deep Sea Drilling Project sites. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are sensitive indicators of diagenetic reactions involving alteration of volcanic matter dispersed in sediments and recrystallization of carbonates. Effects of basalt alteration reactions, except possibly for hydrothermal signals, and of alteration of continental debris are minimal. Carbonate recrystallization has a significant effect on the geochemical mass balance of Sr but diffusion of unradiogenic strontium from interstitial waters has only a small effect on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of ocean water.

IN the study of geochemical cycles much recent attention has been given to evaluating differences in the isotopic compositions of the various geochemical reservoirs. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of marine deposits have been reported extensively and have proven to yield useful isotopic indicators of the provenance of marine sediments¹, the involvement of seawater in the geochemistry of hydrothermal sediments², in basalt alteration³ and in mineral formation⁴. In particular, $^{87}\text{Sr}/^{86}\text{Sr}$ ratios have been used to evaluate the carbonate record of seawater evolution through time^{5–7}.

Less attention has been given to measuring the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of sea waters. Because the current oceanic residence time of Sr ($\sim 10^6$ yr) is much longer than the mixing time of the oceans ($\sim 10^3$ yr) it has been generally assumed that the Sr isotopic composition of seawater is the same everywhere⁷. The value for modern ocean water is close to 0.7092.

However, there are two areas in the marine realm where considerable variability in the dissolved $^{87}\text{Sr}/^{86}\text{Sr}$ ratio can and does occur. One is in seawater hydrothermal systems^{8,9}. The other is in the interstitial waters of marine sediments^{10,11}. Sea waters buried with sediments are subject to major compositional changes because they reflect the diagenetic evolution of the sediments and alteration reactions in underlying basalts^{12,13}. Earlier measurements¹¹ of profiles of the Sr isotopic composition of the interstitial waters at two Deep Sea Drilling Project (DSDP) sites showed decreases in $^{87}\text{Sr}/^{86}\text{Sr}$ ratio with burial

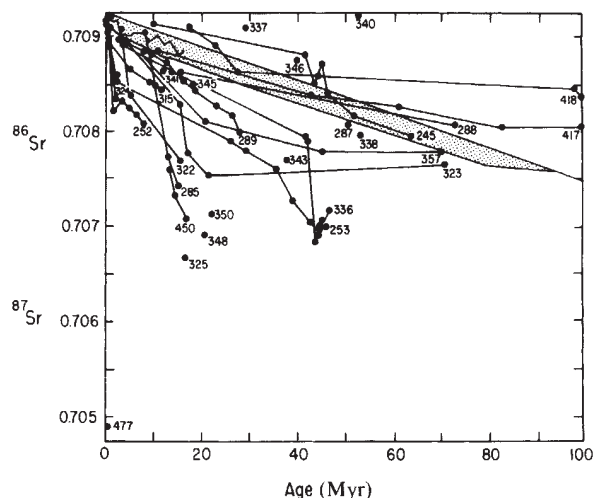


Fig. 1 Plots of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of DSDP interstitial waters versus the age of the sediment sections from which they were extracted. Numbers refer to DSDP sites (Table 1) and the shaded area shows the inferred variation of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of sea water with time and is defined by: (upper) the linear equation of Hawkesworth and Elderfield¹¹ and (lower) the fourth order polynomial of Hart and Staudigel¹⁴; the solid line for 0–16 Myr is from ref. 18.

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depth down to ~0.7069. That study prompted a comprehensive programme of measurement of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in interstitial waters based on samples from, to date, 37 DSDP sites (Table 1) covering the major oceans and a wide range of sedimentary types. Here we summarize the results, the chief generalizations and the inferences which may be made from the whole data set in terms of processes affecting $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, temporal changes, and the geochemical mass balance of Sr isotopes in the oceans.

$^{87}\text{Sr}/^{86}\text{Sr}$ ratios

The Sr isotopic compositions, summarized in Table 1, are plotted in Fig. 1 as a function of the age of the sediment sections from which the interstitial waters were recovered. Examples of the depth profiles are shown in Fig. 2.

Overall, the ratios range from 0.70920 to 0.70904 within 10 m of the sediment surface, values similar to those of contemporaneous sea waters, to a minimum value of 0.70490 in the deepest sample (173 m) obtained from Site 477 in the Gulf of California. With few exceptions the general trend of the data is for $^{87}\text{Sr}/^{86}\text{Sr}$ ratios to decrease from modern-day values as a function of burial depth and age. However, this decrease does not reflect the changing Sr isotopic compositions of seawater^{11,14} because most of the interstitial water values are less radiogenic than contemporaneous sea waters. Such differences cannot be explained purely in terms of diffusive mixing of Sr isotopes within profiles because in many cases the inferred 'age' of the deepest interstitial water sample (using the seawater versus time curve) is greater than the age of the oceanic crust at that site. Even in situations where the age curve for the interstitial waters approximates to that for contemporaneous seawater (for example, Site 245) the Sr isotopic profiles cannot reflect burial of sea waters and no reaction because Sr^{2+} is enriched in the

interstitial waters (Fig. 2). Therefore, the Sr isotopic data must reflect geochemical reactions affecting the compositions of the interstitial waters.

Sources of strontium

The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in potential source materials responsible for the Sr^{2+} enrichment and isotopic compositions of the interstitial waters are¹¹: (1) continental detritus: $^{87}\text{Sr}/^{86}\text{Sr} \sim 0.716$; (2) biogenic carbonates: $^{87}\text{Sr}/^{86}\text{Sr} = 0.7092$ to 0.7075 over 0 to 100 Myr, that is the range for contemporaneous seawaters; (3) volcanic materials (basalts and volcanic ash and glass): $^{87}\text{Sr}/^{86}\text{Sr} = 0.7027$ to ~ 0.705 , the low values for fresh basalts and the high values for materials previously altered by reaction with seawater.

None of the more than 160 interstitial waters analysed have $^{87}\text{Sr}/^{86}\text{Sr}$ ratios which are significantly higher than the ratio for modern seawater, and few samples have ratios which are more radiogenic than contemporaneous sea waters. This rules out continental detritus as a dominant source of the interstitial Sr^{2+} anomalies at most sites. In contrast, the low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of many samples relative to the seawater curve (Fig. 1) show that reactions involving the alteration of volcanic materials must have a major role at many sites.

Geochemical reactions

The chemical and isotopic compositions of the interstitial waters at each site give integrated signals of the combination of diagenetic reactions which, in turn, reflect the mixed lithologies and multiple origins of the sediments. In addition diffusion processes through the interstitial water column will tend to smooth out concentration gradients. Nevertheless, it is possible to identify sites where particular geochemical reactions are dominant.

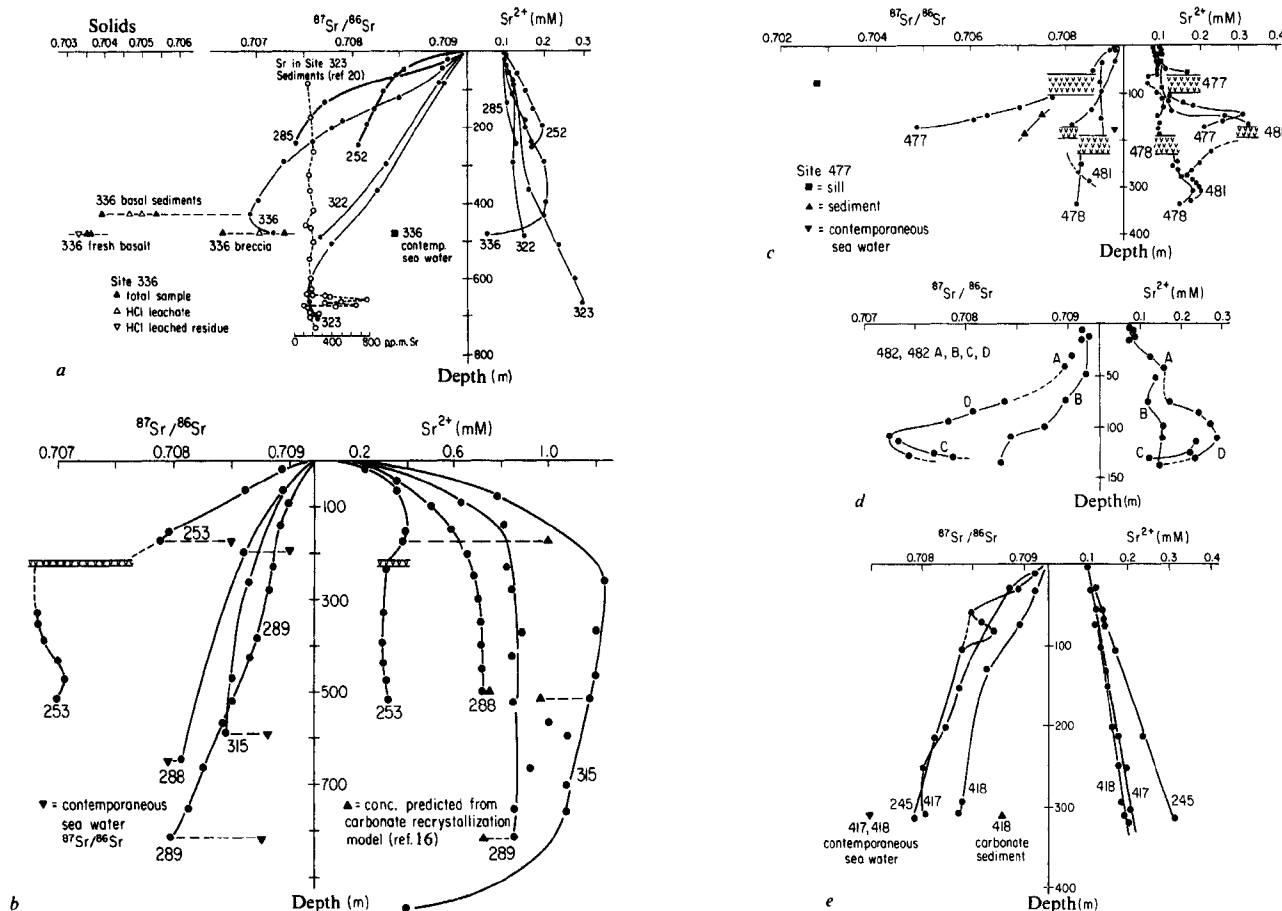


Fig. 2 Depth profiles of $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr^{2+} for DSDP interstitial waters: a, predominantly non-carbonate sites where volcanic material is present in the sediments (Sr concentrations in sediments of Site 323, ref. 20); b, predominantly carbonate sites; c, sites from the Guaymas Basin (the shaded areas represent sills); d, sites from the Tamayo Fracture Zone; e, sites where alteration of continental detritus may be occurring.

We now wish to identify the principal types of reaction that can be inferred from the $^{87}\text{Sr}/^{86}\text{Sr}$ results when considered alongside the Sr^{2+} and other interstitial water data and the locations and lithologies of the sites.

Alteration of volcanic matter: A major feature reflected in Fig. 1 is that in many sites the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of dissolved strontium are much less radiogenic than those of contemporaneous sea waters. The cause of this phenomenon must be sought in substantial contributions to the dissolved strontium pool by reactions involving volcanic matter.

Figure 2a shows profiles from non-carbonate sites containing volcanic matter throughout the sediment column^{11,15,16}. Relatively small enrichments of Sr^{2+} in the interstitial waters are accompanied by large decreases in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios with depth. At Site 323, the moderate enrichment of Sr^{2+} with depth, ~ 0.2 mM over ~ 700 m, was modelled by McDuff and Gieskes¹⁷ in terms of conservative mixing of Sr^{2+} produced from a localized deep source, assumed to be a zone of Danian nanno-chalk and surrounding Sr-rich claystones. The minimum at ~ 650 m in the Sr isotopic profile defines the deep source as this Sr-rich zone and gives a $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of ~ 0.7075 , slightly lower than that for 65-Myr old seawater. The dissolved strontium source, therefore, appears to be derived chiefly from carbonate recrystallization with a small contribution from volcanics. At Site 336, the major Sr^{2+} source is also near-basal sediments. These sediments contain volcanic glass and ash layers are quite common. Comparison of the sediments and associated interstitial waters shows that they are not in Sr isotopic equilibrium.

In all sites presented in Fig. 2a it is apparent that at all depths large deviations occur in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio when compared with contemporaneous seawater (see also Fig. 1). The implication of this is that during alteration of volcanic material dispersed in the sediment column extensive exchange of strontium isotope occurs, without a concomitant large effect on the dissolved Sr^{2+} distribution.

The important question arises whether alteration of basalts of Layer 2 of the oceanic crust also contributes to the Sr^{2+} and $^{87}\text{Sr}/^{86}\text{Sr}$ signals. Such alteration reactions are held responsible for the often observed large increase in Ca^{2+} and decreases in Mg^{2+} , K^{+} and ^{18}O in the overlying interstitial waters^{12,13}. Evidence from dissolved Sr^{2+} profiles (see Fig. 2a) indicates reversals in the $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr^{2+} gradients where near basement was sampled. Results from the base of Site 336 show that Sr isotopic equilibration of the interstitial water and a basaltic breccia zone has occurred whereby the altered rocks have been severely contaminated by seawater Sr. From the Sr contents and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios it seems that the alteration occurred with an effective water-rock ratio of about 20. Thus, although the hydrolysis reaction imparts a Sr isotopic signal to the interstitial water, the resulting $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are not strikingly unradiogenic because of the water-dominated exchange reaction. We surmise that no clear basement signal in $^{87}\text{Sr}/^{86}\text{Sr}$ is observed as a result of high water-rock ratios, and that the major process leading to the low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in interstitial waters is the alteration of volcanic matter dispersed in the sediment column. This, of course, makes this isotopic ratio particularly useful in studies of sediment diagenesis.

Carbonate recrystallization: Although a volcanic Sr signal is undoubtedly present in many of the profiles, the isotopic data cannot be explained simply in terms of the input of strontium to the interstitial waters by dissolution from an unradiogenic source. This is because the profiles which show the least extreme $^{87}\text{Sr}/^{86}\text{Sr}$ gradients show the greatest Sr^{2+} enrichments and vice versa (Fig. 2b). The sites showing large Sr^{2+} enrichments (for example, Sites 315, 289, 288, 253) are all carbonate sites. In these sites, interstitial Sr^{2+} concentrations increase to a concentration maximum, up to over 10 times normal seawater levels (Fig. 2b) and the ratios of Sr^{2+} and Ca^{2+} concentrations, in excess of those of seawater (that is $>7 \times 10^{-3}$) are larger than Sr/Ca in basalts, clays and biogenic carbonate ($\sim 2 \times 10^{-3}$). These enrichments are caused by the release of Sr^{2+} during diagenetic recrystallization of inorganic calcite producing Sr-

depleted chalks and limestones^{18,19,22}. In such cases the interstitial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios will remain close to contemporaneous seawater values provided that active recrystallization is occurring so as to minimize the effect of diffusion.

Site 253 indicates a case where a strong volcanic signal is superimposed on the profile expected from pure carbonate recrystallization. Sedimentation rates in the upper 150 m have been relatively low (~ 4 m Myr⁻¹) and the volcanic sediments below the basaltic sill at 150 m serve as a sink for Sr^{2+} as well as a source of low radiogenic strontium.

Hydrothermal activity: A thermally induced signal in $^{87}\text{Sr}/^{86}\text{Sr}$ has been observed in the interstitial waters at sites in regions of high heat flow on young oceanic crust (Fig. 2c, d). At site 477, located on a large heat flow anomaly (≥ 20 heat-flow units (h.f.u.)) in the southern rift of the Guaymas Basin, Gulf of California, where the bottom-hole temperature is thought to have exceeded 200 °C there is an extremely strong negative $^{87}\text{Sr}/^{86}\text{Sr}$ depth gradient in the interstitial waters down to 0.7049, similar to values for basalt-Sr. The low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are associated with excess ^3He values reflecting hydrothermal processes involving basement rocks²¹. At Site 481, in the northern rift of the basin (~ 4 h.f.u.) the hydrothermal activity must have been associated with the intrusion of a dolerite sill complex which heated the surrounding sediments to as high as ~ 165 °C (ref. 23) and has now cooled to 45 °C.

Though it is tempting to suggest that the low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in these sites are due to hydrothermal alteration of basalts, it is equally possible that alteration of volcanic debris has caused the $^{87}\text{Sr}/^{86}\text{Sr}$ decreases near the sills. This is demonstrated well in Site 482 in the Tamayo Fracture Zone²⁴, where the high heat flow (12 h.f.u.) has apparently accelerated the rate of alteration of volcanic matter in the sediments producing

Table 1 Summary of Sr isotopic compositions

⁸⁷ Sr/ ⁸⁶ Sr					
Site*	Location		No. of samples	Range	Ref.
245	31°32' S	52°18' E	8	0.70913–0.70794	11
252	37°3' S	59°14' E	7	0.70926–0.70807	†
253	24°53' S	87°22' E	9	0.70893–0.70683	†
285	26°49' S	175°48' E	4	0.70903–0.70742	†
287	15°55' S	153°16' E	1	0.70805	†
288	5°59' S	161°49' E	2	0.70861–0.70807	18
289	0°30' S	158°31' E	13	0.70918–0.70798	18
315	4°10' N	158°32' W	5	0.70904–0.70843	†
322	60°02' S	79°25' W	3	0.70892–0.70767	†
323	63°41' S	97°60' W	5	0.70897–0.70755	†
324	69°03' S	98°47' W	2	0.70890–0.70831	†
325	65°03' S	73°41' W	1	0.70667	†
336	63°21' N	7°47' W	10	0.70916–0.70694	11
337	64°52' N	5°21' W	1	0.70911	†
338	67°47' N	5°23' W	1	0.70794	†
339	67°13' N	6°17' E	1	0.70899	†
340	67°13' N	6°18' E	1	0.70923	†
341	67°20' N	6°7' E	1	0.70864	†
342	67°57' N	4°56' E	1	0.70898	†
343	68°43' N	5°46' E	1	0.70769	†
345	69°50' N	1°14' W	1	0.70851	†
346	69°54' N	8°41' W	1	0.70875	†
348	68°30' N	12°28' W	1	0.70691	†
349	69°13' N	8°6' W	1	0.70862	†
350	67°03' N	8°18' W	1	0.70713	†
357	30°00' S	35°34' W	4	0.70907–0.70777	†
417	25°07' N	68°03' W	6	0.70924–0.70802	†
418	25°02' N	68°04' W	5	0.70910–0.70836	†
450	18°00' N	140°48' W	3	0.70762–0.70708	†
474	22°57' N	108°59' W	11	0.70916–0.70765	21
476	23°03' N	109°06' W	5	0.70915–0.70866	21
477	27°02' N	111°24' W	9	0.70891–0.70490	21
478	27°06' N	111°31' W	5	0.70875–0.70822	21
479	27°51' N	111°38' W	6	0.70920–0.70894	21
481	27°15' N	111°31' W	7	0.70905–0.70816	21
482	22°48' N	107°60' W	19	0.70921–0.70733	24
503	4°04' N	95°38' W	4	0.70919–0.70903	†

* For information on DSDP sites, see the appropriate volume of *Initial Reports of the Deep Sea Drilling Project* (US Government Printing Office, Washington DC).

† Unpublished results: data report available on request to H.E.

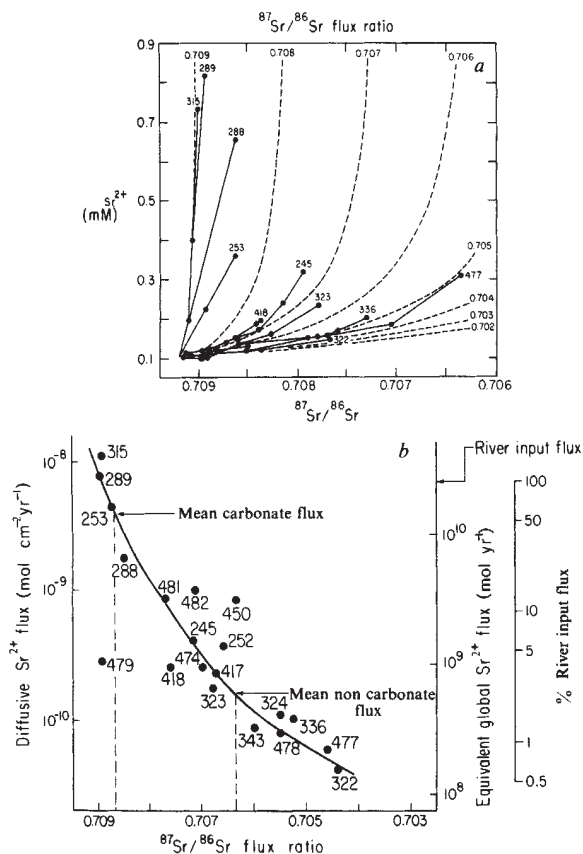


Fig. 3 *a*, Plots of Sr^{2+} against $^{87}Sr/^{86}Sr$ for uppermost sections of interstitial water profiles. Numbers refer to DSDP sites and the curves labelled with $^{87}Sr/^{86}Sr$ ratios are the ratios of fluxes of ^{87}Sr to ^{86}Sr , calculated from: $flux(^{87}Sr)/flux(^{86}Sr) = D(\Delta^{87}Sr/\Delta \text{ depth})/D(\Delta^{86}Sr/\Delta \text{ depth})$, where D is diffusion coefficient. *b*, Diffusive Sr^{2+} flux against $^{87}Sr/^{86}Sr$ flux ratio. Sr^{2+} flux calculated from: $D\Delta^{87}Sr/\Delta \text{ depth}$ where $D = 2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (ref. 27); flux ratio calculated as above. Numbers refer to DSDP sites. Also shown are average carbonate and non-carbonate fluxes (see text) and river input flux⁷. The equivalent global flux is obtained using the oceanic area of $361 \times 10^{16} \text{ cm}^2$.

pronounced extrema in $^{87}Sr/^{86}Sr$ and dissolved strontium. The isotopic signal is stronger in Holes 482C and D where the thermal influence is thought to have been greater than in Holes 482A and B and, overall, the isotopic changes occur at shallower depths and during earlier diagenesis than at the sites shown in Fig. 2*a* where high temperatures were not experienced. Such $^{87}Sr/^{86}Sr$ changes appear to be associated with temperature-dependent silicification reactions involving the alteration of volcanic matter in the sediments and the diagenesis of opaline silica giving rise to 'diagenetic fronts' in the sediments at depths which relate to the thermal influence at each site²⁴⁻²⁶.

Diagenesis of continental debris: Rarely do the $^{87}Sr/^{86}Sr$ profiles show evidence of reaction which may be attributed to material of continental origin having radiogenic Sr isotopic compositions. At Site 245 (Fig. 2*e*) the interstitial waters at ~75 m are distinctly more radiogenic than contemporaneous seawater which may indicate exchange reactions involving continental debris. At Sites 417 and 418 the $^{87}Sr/^{86}Sr$ ratios of the interstitial waters are everywhere significantly higher than for contemporaneous seawaters (Fig. 2*e*) and persist to close to basement into a narrow zone of impure Cretaceous carbonates contaminated with continental detritus which appears to be the main Sr^{2+} source. However, these sites are characterized by

very slow sediment accumulation rates ($\sim 3 \text{ m Myr}^{-1}$) and diffusive communication with the overlying ocean will exist throughout the pore water profiles. Thus, the high $^{87}Sr/^{86}Sr$ ratios may reflect diffusive mixing of Sr-isotopes. Similar arguments could be made for Sites 337 and 340 (Fig. 1). Therefore, no sites yet studied show unequivocal evidence of a Sr-isotopic signal reflecting diagenesis of continental debris.

The above discussion reveals that the major processes affecting the $^{87}Sr/^{86}Sr$ distribution in interstitial waters of deep sea sediments are the alteration of volcanic matter dispersed in the sediments and the recrystallization of carbonates. Even in drill sites consisting almost of pure carbonate sediments a volcanic signal is often superimposed on that expected from carbonate recrystallization processes. Effects of alteration reactions involving basalts of Layer 2, with the possible exception of hydrothermal signals, and of the alteration of continental debris are minimal.

Geochemical mass balance

One of the major aims of the DSDP interstitial water programme has been to evaluate the exchange of chemicals between the oceans and the sediments and basalts of oceanic crust. It has been shown that the diffusive fluxes of the major constituents Ca^{2+} , Mg^{2+} and K^+ , as deduced from interstitial waters concentration gradients, do not have a significant role in the geochemical mass balance of the oceans whereas the oxygen isotopic flux may be of importance^{12,13}.

The Sr^{2+} concentration gradients shown in Fig. 2 are about 0.5–1.0 mM per 100 m for carbonate sites and about 0.02–0.03 mM per 100 m for non-carbonate sites where volcanic matter is undergoing alteration. With an average diffusion coefficient of $2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (ref. 27), and neglecting advection, the values are equivalent to integrated global fluxes of the order of $1\text{--}2 \times 10^{10} \text{ mol yr}^{-1}$ for carbonate sites and $5\text{--}7 \times 10^8 \text{ mol yr}^{-1}$ for non-carbonate sites, as compared with the river input flux of $2.5 \times 10^{10} \text{ mol yr}^{-1}$ (Fig. 3*b*). Assuming that carbonate sediments occupy 25% of the sea floor one calculates an average Sr flux from carbonate sites of ~20% of the river flux. The flux from non-carbonate sites is trivial, globally ~2% of river input.

The effect of these interstitial Sr^{2+} fluxes on the Sr isotopic balance of the oceans is small. This is because the largest interstitial Sr^{2+} gradients (and, hence, the largest fluxes) are associated with smallest $^{87}Sr/^{86}Sr$ depth gradients and vice versa. A combination of Sr^{2+} and $^{87}Sr/^{86}Sr$ data allows the diffusive flux ratios of ^{87}Sr to ^{86}Sr to be defined using the ratios of their concentration gradients versus depth (Fig. 3). Figure 3*b* shows a very consistent pattern for nearly all sites with high values of the $^{87}Sr/^{86}Sr$ flux ratio associated with high Sr^{2+} fluxes and low values of the isotopic flux ratio associated with low total fluxes. The large average Sr^{2+} flux from carbonate sites has an average $^{87}Sr/^{86}Sr$ ratio of ~0.7087, close to the modern seawater ratio as compared with non-carbonate sites which have an average $^{87}Sr/^{86}Sr$ ratio of ~0.7064. However, the latter have a lesser effect on the oceanic Sr isotope budget because the associated Sr^{2+} flux is an order of magnitude smaller. Overall, the effect is small and of minor significance in the oceanic budget of Sr-isotopes^{8,9}. This evaluation does not, of course, include the transport by advection of interstitial waters with anomalous $^{87}Sr/^{86}Sr$ ratios. It has been suggested^{8,9} that the flux of hydrothermal fluids supplied to the ocean by advection plays a significant role in fixing the $^{87}Sr/^{86}Sr$ ratio of seawater. The advection of interstitial waters with low $^{87}Sr/^{86}Sr$ ratios because of the influence on hydrothermal processes would add to this flux.

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Oxygen isotope evidence for shallow emplacement of Adirondack anorthosite

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Oxygen isotopic analysis of wollastonites from the Willsboro Mine, Adirondack Mountains, New York reveals a 400-ft wide zone of ^{18}O depletion at anorthosite contacts. Values of $\delta^{18}\text{O}$ vary more sharply with distance and are lower (to -1.3) than any yet reported for a granulite facies terrain. Exchange with circulating hot meteoric water best explains these results and implies that the anorthosite was emplaced at relatively shallow depths, <10 km, in marked contrast to the depth of granulite facies metamorphism (23 km). These ^{18}O depletions offer the first strong evidence for shallow emplacement of anorthosite within the Grenville Province and suggest that regional metamorphism was a later and tectonically distinct event.

MASSIF-TYPE anorthosite is an important rock type in the Grenville Province and in many other deeply eroded Precambrian terrains of US–Canada. Most of these bodies were metamorphosed in granulite facies conditions that imply burial depths of 20–30 km (refs 1–3). This observation is commonly used as evidence that anorthosite selectively intrudes and solidifies in the lower crust and thus may be a major constituent of a mafic layer below the Conrad seismic discontinuity.

In the Adirondack Mountains, New York and elsewhere petrological evidence has been used to suggest that anorthosite intruded at shallow depths before deep burial and metamorphism. Buddington⁴ interpreted the borders of the Adirondack Massif as a chill margin with massive contact skarns and therefore proposed that it intruded shallowly into comparatively cool country rocks. The presence of wollastonite, an index mineral of the sanidinite facies^{5,6}, supported this view⁷. However, wollastonite has now been recognized in many amphibolite and granulite facies terrains^{2,8–11} indicating that the classical assumption that $P_{\text{lithostatic}} = P_{\text{CO}_2}$ is not valid for the metamorphism of all marbles^{2,11,12}. Thus the petrological evidence is ambiguous, permitting either shallow or deep intrusion^{13–15}.

We have studied the C–O–H stable isotope geochemistry and phase equilibria of contact skarns in the Adirondacks. While phase equilibria are inconclusive as to depths of emplacement, oxygen isotope studies at the Willsboro Mine, a massive skarn, provide new data that bear on this important problem.

The Willsboro Mine

The Willsboro Mine, an important source of wollastonite since 1940, is located in a 0.25-km thick skarn belt at the contact of the Port Kent–Westport unit of the main Adirondack anorthosite massif. This skarn belt extends over 7 km in the Willsboro quadrangle and may continue an additional 8 km into the Au Sable quadrangle, connecting two other wollastonite deposits at Deerhead and Lewis, New York. The anorthosite forms a structural trough in the region of this belt and shows evidence of extensive magmatic assimilation of meta-sediment, including common xenoliths of marble and evolved anorthosite compositions^{4,16}. Similar features are found on mountain tops in adjacent quadrangles, indicating that

originally overlying metasediments were engulfed in the roof zone of the anorthosite body.

The wollastonite ore-rock is distinctively similar throughout these three deposits, consisting of parallel layers of three coarse grained minerals: wollastonite, clinopyroxene, and grandite garnet. Compositional variation is limited and of major-element solid solutions, only Fe/Mg and Fe/Al vary among the pyroxenes and garnets of different layers. At Willsboro two major and many minor layers of ore-rock dip 30–55° NNE with anorthosite forming the footwall of the deposit. Surrounding and between these layers of ore-rock are layers of mixed gneisses including mafic gneiss, amphibolite, and granitic gneiss.

All rocks in the Adirondacks, except a few late dykes, show the effects of a pervasive metamorphism that occurred during the Grenville orogeny. At Willsboro and elsewhere in the north-east Adirondacks, the maximum pressure and temperature of granulite facies equilibration are estimated from many mineral thermometers and barometers to be 7 ± 1 kbar and $700 \pm 50^\circ\text{C}$ (refs 1, 3, 17, 18). U/Pb, Rb/Sr and Nd/Sm ages indicate that metamorphic recrystallization overprinted earlier igneous events^{19–21}. Various age estimates suggest that the anorthosite intruded at 1,200 Myr, at least 100 Myr before the peak of metamorphism, but it cannot be resolved whether igneous activity was a precursor of metamorphism with intruding magmas contributing heat to raise the geotherm, or whether intrusion and metamorphism represent two discrete, unrelated events.

The Willsboro skarn belt gives clear evidence of a metasomatic origin. The wollastonite ore-rock consistently contains three phases and five components for 4 degrees of freedom, and it is commonly reported to be totally devoid of any calcite or quartz²². We have located a few thin layers containing calcite at Willsboro (see Table 1), but these amount to only a few centimetres out of thousands of metres of drill core that were examined. If wollastonite formed through isochemical metamorphism (with only fluid components added or subtracted) through the reaction:



the entire original limestone unit would have had to contain

an impossibly perfect molar ratio of 1 calcite to 1 quartz. Thus large amounts of material were either removed or added in solution, indicating that massive fluid circulation was involved in the ore genesis.

Stable isotope results

Analysis of mineral separates from 18 samples of drill core from a 700 ft traverse of the Willsboro Mine (at 7,600 W, mine coordinates) shows that unusually low values of $\delta^{18}\text{O}$ (2.9 to -1.3) are restricted to localities within 400 ft of the anorthosite contact. Taylor²³ reported a similar low value of -0.9 for one sample of wollastonite from Willsboro. These low values form a steep-sided trough (Fig. 1) with sharp gradients to higher $\delta^{18}\text{O}$ in the gabbroic anorthosite of the footwall and in the mafic gneisses of the hanging wall. The magnitude of this trough is normalized in Fig. 1 by a line connecting wollastonite analyses or, in the case of rocks not containing wollastonite, through the value of a fictive wollastonite, calculated assuming equilibrium isotopic fractionations at 700°C (refs 24, 25).

Other wollastonite samples from the main layers of ore-rock at Willsboro as well as from Lewis and Deerhead (Table 1) have similarly low $\delta^{18}\text{O}$ values (3.1 to -1.2) suggesting that the entire 15-km skarn belt is ^{18}O -depleted near the anorthosite contact. We are making further analyses to test this hypothesis, as it would indicate that the ^{18}O -depleting event affected many cubic kilometres of rock.

Three coexisting minerals were analysed from a sample of Willsboro ore-rock (82-W-1) to test that equilibrium isotopic values^{24,25} have been preserved from the peak of regional metamorphism (Table 1). These mineral analyses approximate the values that are expected at 700°C considering that the fractionations and chemical compositions are not well known. The large ^{18}O anomaly cannot have formed by retrograde exchange with post-metamorphic fluids as such an exchange would differentially affect the different minerals.

The gradients in this isotopic trough are the sharpest yet reported for non-carbonate-bearing rocks from any granulite facies terrain. In drill hole DDH 50, $\delta^{18}\text{O}$ values vary by 8.0‰ in 14 ft between ore-rock and amphibolite and by 6.0‰ in 3 ft within ore-rock. Additional sampling and analysis will elucidate the details of these gradients. In this respect the apparently parallel layers of the Willsboro deposit cause problems. In spite of extensive drilling and mining it is not now known to what extent these gradients are due to tectonic juxtaposition through faulting, recumbent folding, or deformation. Faulting, in particular, could cause gradients in $\delta^{18}\text{O}$, and the coarse regional metamorphic recrystallization of the deposit must be considered, as it could effectively conceal evidence of such move-

ments if they were premetamorphic. We have not seen any textural evidence of faulting in the zones of high $\delta^{18}\text{O}$ gradient, nor are indications of faulting reported by previous workers. Thus if tectonic processes are responsible for these gradients, they operated during an earlier event before peak metamorphic recrystallization, and these gradients were then preserved through the granulite facies metamorphism.

Seven samples of amphibolite were also analysed for hydrogen isotopic composition. Unfortunately the measured δD values of -86 to -68 fall in a range commonly observed in igneous, metamorphic and sedimentary rocks and are not indicative of the origin of fluids involved in the genesis of this deposit.

Cause of the ^{18}O depletion

Large ^{18}O depletions occur at igneous contacts by only two processes: metamorphic volatilization and exchange with hot meteoric water. Estimates of the effects of volatilization, however, indicate that they are insufficient to account either for the extreme magnitude of ^{18}O depletion (up to -20%) or the extreme size (cubic kilometres) of the Willsboro skarn belt.

Unusually low $\delta^{18}\text{O}$ values have also been reported from granulites of widely separated portions of the Strangways Range, Australia (1.8‰ for hypersthene, 2.8‰ for cordierite)²⁶. Additional mechanisms for ^{18}O depletion have been proposed for these rocks including premetamorphic diagenesis and high temperature metamorphic exchange with deep-seated mafic reservoirs. These processes cannot have been important at Willsboro as the ^{18}O anomaly is restricted to the skarn belt and is not seen in any of 85 regionally distributed marble samples that have normal values of $\delta^{18}\text{O}$ (ref. 27).

Volatilization can be considered as the slow release of CO_2 due to the reactions such as (1). The magnitude of ^{18}O depletion is then largest for reaction in shallow terrains, in spite of the pressure independence of equilibrium isotope fractionations, because temperatures of reaction will be lowest at low pressures, resulting in higher enrichments of ^{18}O in the escaping CO_2 . We have made equilibrium calculations at 300–600°C based on the stoichiometry of well-known, idealized reactions for the complete decarbonation of a siliceous marble. These calculations suggest that the extremes of ^{18}O depletion for regional and contact metamorphism are approximately 5 and 7‰, respectively. These ^{18}O depletions would be even less if decarbonation is not complete, if non-reacting minerals are present in the rock, if H_2O or CH_4 dilute the metamorphic CO_2 , or if degassing is not controlled by Rayleigh distillation²⁸ with equilibrium fractionations. If isotopic fractionations are not equilibrium fractionations, then the magnitude of ^{18}O depletion due to volatilization will again be reduced. Experiments on the rapid, non-equilibrium, thermal decomposition of dolomite show that the evolved CO_2 is only slightly enriched in ^{18}O relative to dolomite²⁹.

Results from the Mottled Zone, Israel, should represent an extreme case of volatilization depletion. In response to heat from naturally burning organic matter, calcite completely reacted with silicates at depths of <100 m, and the measured ^{18}O depletions are generally $<7\%$ (ref. 30). Similar results are observed for low pressure contact metamorphism at Marble Canyon, Texas, where a difference of 9‰ (normalized to wollastonite) was found between adjacent zones of calcite + wollastonite and spurrite³¹. Some ^{18}O depletion in these areas could have been caused by hot circulating groundwaters, and thus these values may be viewed as upper limits. Numerous other contact aureoles have been shown to be less affected by volatilization^{31–35}.

In the Adirondacks analyses of 85 carbonates average $\delta^{18}\text{O} = 20$ (ref. 27). This is in keeping with the average $\delta^{18}\text{O}$ value of 21 for worldwide unmetamorphosed carbonates of the same 1,200 Myr age³⁶. In contrast the Willsboro wollastonite is depleted by $>20\%$. This ^{18}O depletion is 3–5 times larger, depending on depth of metamorphism, than is found at other documented localities or than our calculations indicate is possible through volatilization.

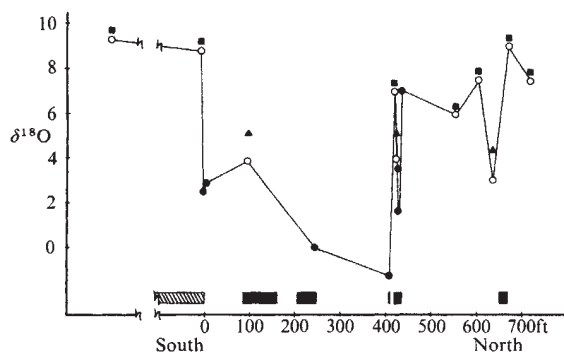


Fig. 1 Values of $\delta^{18}\text{O}$ for minerals from a 700-ft traverse of the anorthosite contact with wollastonite skarn at the Willsboro Mine. The 400-ft wide trough of ^{18}O depletion includes the lowest $\delta^{18}\text{O}$ (-1.3) and the sharpest $\delta^{18}\text{O}$ gradients known from a granulite facies terrain. Plagioclase in the hanging-wall anorthosite (south) is isotopically similar to other Adirondack anorthosites. ●, Wollastonite (measured); ○, wollastonite (calculated); ■, plagioclase; ▲, calcite; solid shading, wollastonite ore-rock; and hatching, anorthosite.

Table 1 Values of $\delta^{18}\text{O}$, δD and $\delta^{13}\text{C}$ for mineral separates from the Willsboro Skarn Belt

Sample no.	Description	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	δD	Sample no.	Description	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	δD
AUS 78-1	Plagioclase megacryst from anorthosite, Lewis Quarry	9.7			DDH W 50-425'	Plagioclase from amphibolite whole rock	9.3		-86.2
AUS 78-4	Wollastonite from ore-rock, Lewis Quarry	-1.2			DDH W 50-475'	Plagioclase from amphibolite	7.8		
81-AUS-1	Wollastonite from ore-rock, Deerhead Quarry	1.2			W 77-100	biotite from amphibolite			-78.0
W 2-3b	Calcite from marble 2.5 km ENE of Willsboro Mine	21.9	1.1		W 77-103	Plagioclase from foot-wall mafic gneiss in outcrop at no. 4 portal at Willsboro Mine	9.8		
DDH W 50-1'	Wollastonite from ore-rock in main ore zone, DDH 50 at 1-ft level, Willsboro Mine	0				Plagioclase from hanging wall amphibolite in outcrop at no. 2 portal at Willsboro mine	8.6		
DDH W 50-163'	Wollastonite from ore rock, 3-ft layer within amphibolite	-1.3			DDH W 81-3-5'	hornblende	5.8		-86.0
DDH W 50-177'	Plagioclase from amphibolite	6.7			DDH W 81-3-88'	Calcite from 5-cm thick layer of calcite+clinopyroxene+garnet	5.1	-3.6	
	Hornblende from amphibolite			-69.6	DDH W 81-3-91'	Wollastonite from 1.3-m thick layer of ore-rock	2.9		
DDH W 50-178'	Calcite from 2-cm thick layer of calcite+clinopyroxene+scapolite	5.5	-1.4		DDH W 81-3-91'	Wollastonite from same layer as -88'	2.5		
DDH W 50-181'	Wollastonite from ore-rock, 19-ft layer within amphibolite	3.5			DDH W 81-3-101'	Plagioclase phenocryst from gabbroic anorthosite	9.3		
DDH W 50-183'	Wollastonite from ore-rock	1.0			DDH W 81-7-1'	Wollastonite from ore-rock in foot wall 1,200 ft ESE of DDH W 81-3	3.1		
DDH W 50-186'	Wollastonite from ore-rock	7.0			DDH W 81-9-94'	Plagioclase phenocryst from gabbroic anorthosite 200 ft WNW of DDH W 81-3	7.8		
DDH W 50-216'	Biotite from amphibolite			-75.4	81-W-21	Plagioclase phenocryst from anorthosite 2 km E of the Willsboro Mine	9.1		
DDH W 50-287'	Biotite from amphibolite			-73.3		Coexisting minerals from ore rock, Willsboro Mine			
DDH W 50-307'	Plagioclase from amphibolite	6.3				wollastonite	-0.7		
	hornblende	4.8				clinopyroxene	0.3		
DDH W 50-351'	Biotite from amphibolite			-68.5	82-W-1	garnet	-0.5		
DDH W 50-357'	Plagioclase from amphibolite	7.9							
DDH W 50-391'	Calcite from 10-cm thick layer of calcite+garnet+clinopyroxene	4.4	-2.2						

If the ^{18}O depletion at Willsboro resulted from volatilization of CO_2 then a complimentary ^{13}C depletion should be seen. Carbonates from contact aureoles that have undergone variable amounts of volatilization often show a direct correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ for this reason³⁰⁻³⁵. The ratio of ^{13}C depletion to ^{18}O depletion is controlled by the relevant CO_2 -mineral fractionations and the mechanism of volatilization, but ^{13}C depletion is always at least one-third that of ^{18}O . Three calcites from thin lenses (2–10 cm thick) within the Willsboro ore-zone have an average $\delta^{13}\text{C} = -2.4$. This is 2.5% less than the average of 75 Adirondack calcites (0.1)²⁷ indicating that the magnitude of ^{18}O depletion at Willsboro is $\leq 7.5\%$, in accord with our estimate based on calculations and other field studies.

Exchange with heated meteoric waters is a well documented process, causing massive depletions of up to 15% to $\delta^{18}\text{O}$ values below -10 at the margins of cooling plutons where convective fluid flow facilitates high water-to-rock ratios³⁷. Exchange with meteoric water is thus the most probable cause of ^{18}O depletion, especially given the association of wollastonite with the roof zone of the anorthosite massif.

It is common for hot circulating meteoric waters to lower the ^{18}O content of the solidified margins of a pluton as well as the surrounding country rock. In the Adirondacks, the $\delta^{18}\text{O}$ values of plagioclase in anorthosite (8.9–11.2) are generally 3% higher than anorthosites and gabbros elsewhere. However, five unusually garnet-rich anorthosites have low $\delta^{18}\text{O} = 4.3$ to 5.7 (ref. 23). Regardless of whether the general ^{18}O enrichment

occurred during the metamorphic²³ or magmatic³⁸ event, some additional process is necessary to explain these garnet-rich anorthosites that are lower in ^{18}O than normal anorthosite. These samples are from near the contacts of the anorthosite and have been interpreted as resulting from metasomatic interaction with metasedimentary wall rock^{4,16}. It is reasonable to suggest that these anorthosite samples have also interacted with meteoric water.

Shallow intrusion of anorthosite

The involvement of large amounts of meteoric water in the skarn formation at Willsboro would require a depth of anorthosite emplacement much less than the 23 km inferred for granulite facies metamorphism. This is because meteoric waters circulate at hydrostatic pressures (P_H) and cannot penetrate to depths of high grade metamorphism, where porosity is extremely low and fluids are at lithostatic pressures ($\sim 3P_H$). The maximum depths to which groundwater circulates are estimated^{39,40} at 7–10 km. Many documented examples of ^{18}O exchange with meteoric waters occur at the margins of such shallowly emplaced plutons⁴¹, and we propose that the Adirondack massif likewise intruded at shallow depths of <10 km.

Shallow anorthosite intrusion is supported by the presence of contact aureoles at two anorthosite massifs from outside of the Grenville Province. At Nain coexisting orthopyroxene + olivine + quartz limits pressure^{3,13} to 3.2 ± 0.2 kbar, and at Laramie coexisting pigeonite + hedenbergite + fayalite limits pressure⁴² to 1.5–2.0 kbar. These bodies lie to the north-west

of the Grenville Front and do not show Grenville metamorphic overprints. It is thus likely that North American anorthositic intrusions at all levels in the crust before the Grenville orogeny and that subsequently those nearest the continental margin were buried at the time of metamorphism. Such rapid burial is supported in the Adirondacks by whole-rock Rb/Sr ages of $1,263 \pm 25$ Myr for surface eruption of ash-flow tuffs that were subsequently metamorphosed (1,120 Myr) at 20 km depth to leuco-gneiss²¹. The common inference may thus be incorrect and the close association of anorthosite with granulite facies metamorphism seems to have no relevance to actual emplacement depths.

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We believe that these Adirondack results are best explained by the following events. Anorthositic magma intruded to shallow depths at $\sim 1,200$ Myr. Metasomatism and skarn formation took place during solidification and cooling in the roof-zone with the involvement of large amounts of low- $\delta^{18}\text{O}$ meteoric water. The anorthosite and skarn were subsequently buried to 23 km and metamorphosed at $\sim 1,100$ at low f_{CO_2} .

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Evidence for the direct involvement of DNA replication origin in synthesis of late SV40 RNA

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Analysis of the late simian virus 40 (SV40) transcription initiation patterns of viable and non-viable mutants and a DNA replication-negative mutant in permissive monkey cells and/or in Xenopus laevis oocytes suggests a direct role for the origin of DNA replication in the activation of late transcription.

THE SV40 genome contains two transcription units. The early unit is functional in permissive cells throughout the infection cycle and in SV40-transformed cells. The minimal promoter elements and more remote regulatory functions have been characterized in detail^{1–4}. The late transcription unit, however, is actively transcribed only in permissive cells during the late phase of the infection cycle. Late mRNA is not synthesized in significant quantities in transformed cells, in lytically infected cells in the presence of inhibitors of DNA synthesis^{5,6}, or in cells infected at the non-permissive temperature with temperature-sensitive (ts) A mutants^{6,7}. Furthermore, microinjection experiments with form I and replicative intermediate DNA into chemically blocked permissive cells or non-permissive cells, also suggested a role of DNA replication in late mRNA synthesis^{8,9}. However, other data obtained from studies with transcription complexes^{10,11}, early mRNA¹², or mRNA from infected non-permissive cells¹³, led to the rejection of the idea of a special role for DNA replication in the activation of the late SV40 promoter. These studies suggest that the large amount

of late mRNA is caused by a marked increase in the gene copy number. The much smaller amount of early RNA observed at late times after infection may be explained by the autoregulatory function of large T antigen^{14,15}.

We attempted to resolve this controversy and to give a better definition in molecular terms of the requirements for synthesis of late SV40 mRNA. We therefore analysed the RNA produced by several deletion mutants in the putative late promoter region¹⁶ (D. G. *et al.*, in preparation) and a DNA replication-negative mutant in infected CV1 cells and/or in *X. laevis* oocytes. Extensive deletions in the late initiation region were found to have a very limited effect on downstream initiation points, but a deletion of four base pairs (bp) that had been shown to prevent DNA replication¹⁷ caused a marked inhibition of late RNA synthesis even at some downstream initiation points over 300 nucleotides away.

5'-Termini of wild-type and deletion mutant late mRNAs in CV1 cells

The 5'-termini of late lytic SV40 RNAs are strikingly heterogeneous^{18–20}. The positions of these 5'-termini have been mapped by reverse transcription¹⁸ and by direct analysis^{19,21}.

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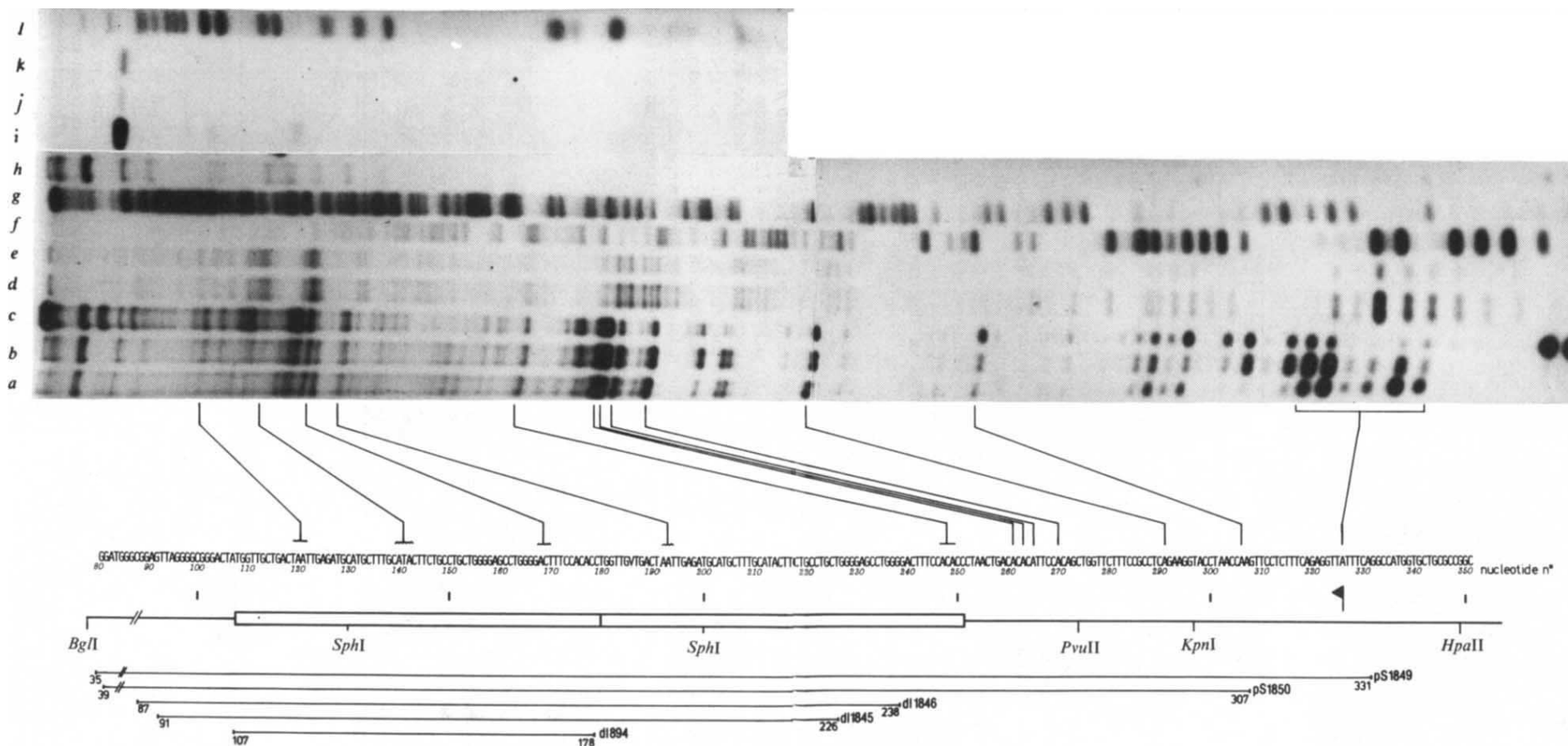
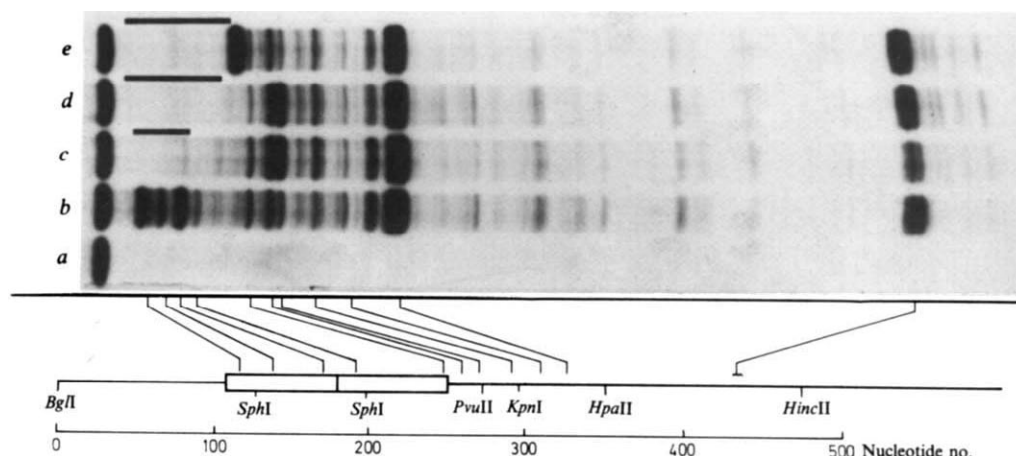


Fig. 1 Mapping of 5' termini of SV40 late mRNA with S_1 nuclease and exonuclease VII. The upper part of the figure shows a 12% polyacrylamide-8 M urea gel of wild-type (wt) SV40 late mRNA 5'-termini. (Migration in all the figures is from left to right.) 1 μ g of poly (A)⁺ RNA isolated from SV40-infected CV1 cells 48 h after infection as described elsewhere¹⁹, was hybridized to a single-stranded (SS), 5'-end-labelled *HpaII*-*MboII* DNA probe, and digested with exonuclease VII at increasing concentrations of NaCl (a, 50 mM; b, 200 mM; c, 300 mM) or with S_1 nuclease (d, e) as described elsewhere^{22,45} (d and e are identical experiments carried out separately). The 5'-labelled restriction fragments in this and all following experiments were strand-separated on a 5% polyacrylamide gel as described⁴⁶, and an aliquot of the isolated single strand was co-precipitated with each RNA preparation. After hybridization in 15 μ l buffer (40 mM PIPES pH 6.4, 400 mM NaCl, 1 mM EDTA) for a minimum of 4 h, the hybrids were digested with S_1 nuclease or exonuclease VII. Lanes f (T+C) and g (A+G) show a Maxam-Gilbert⁴⁶ DNA sequence ladder. Lane h is a control digest of the probe. Lanes i-k are S_1 nuclease digests in conditions identical to those of d and e with strands separated from the SV40 *Hind* F RI fragment. As this fragment is localized in the body of the VP1 gene, the late coding strand is entirely protected against S_1 nuclease digestion (lane i); the other strand is not protected (lane j). Lane k shows the late coding strand digested in the absence of RNA and lane l is a chain length marker. This experiment (lanes i-l) was carried out on a 20 $\times$ 40 cm polyacrylamide sequencing gel, while lanes a-h were fractionated on a 30 $\times$ 90 cm gel. The lower part of the figure shows the nucleotide sequence of the region where the late SV40 mRNAs initiate. The initiation points as deduced from a comparison of lanes a-d with lanes f and g (and considering data in the literature; see text) are indicated. The identification of the exact 5' ends of the four longest bands (wavy lines) is less accurate than for the others; they were assigned from a longer run (results not shown). Some characteristic restriction sites and the 72-bp repeats are also shown. The flag indicates the major SV40 wt initiation of late mRNA at position L325. The positions of the deletion mutants are indicated at the bottom; the deletions are identified by the nucleotide numbers (the numbering system is as described previously^{47,48}). The deletion mutants were constructed as follows: pBR325 was deleted for the tetracycline gene by partial *HaeII* digestion, which produced the plasmid pBGF1 (D. G. *et al.*, in preparation). Wild-type SV40 and dl 894 DNA were digested with *EcoRI* and ligated in the unique *EcoRI* site of the chloramphenicol resistance gene. After opening at the *SphI* site in the cloned SV40 DNA, treatment with nuclease *Bal31*, followed by re-ligation, produced the series of deletions which were characterized by nucleotide sequence analysis.

Fig. 2 Exonuclease VII mapping of the 5'-ends of wt- and viable mutant-induced late poly(A)⁺ mRNA extracted from CV1 cells. Cytoplasmic poly(A)⁺ mRNA of wt and mutant viruses extracted at 48 h post-infection was hybridized against the single-stranded [5'-³²P]-labelled *Bgl*II-*Hind*II (position 472) fragment and the RNA-DNA hybrid digested with exonuclease VII as described in Fig. 1 legend. *a*, 1 µg poly(A)⁺ mRNA from uninfected CV1 cells; *b*, 1 µg poly(A)⁺ mRNA of wt-infected CV1 cells; *c*, 0.5 µg poly(A)⁺ mRNA from dl 894-infected cells; *d*, 1.0 µg poly(A)⁺ mRNA from dl 1845-infected cells; *e*, 1.0 µg poly(A)⁺ mRNA from dl 1846-infected cells. The size and position of the deletion is shown on the polyacrylamide-urea gel by a solid line (see also diagram in Fig. 1). The lower part of the figure is a schematic representation of the wt initiation points and major restriction enzyme sites.



Previously we have shown that the physical 5' cap structures correspond to genuine initiation points for transcription¹⁶. For the present analysis of the late mRNAs, we used the *S*₁ nuclease and/or exonuclease VII mapping technique with single-stranded probes [5'-³²P]-labelled at the *Hpa*II (Fig. 1) or *Hind*II sites (Fig. 2). An *S*₁ nuclease and an exonuclease VII 5'-end analysis of wild-type mRNA are shown in Fig. 1. As in a high resolution gel, three to five bands are formed per 5'-end after an *S*₁ digestion, the pattern obtained is rather complex and is not very well suited for mapping at the exact nucleotide level especially in regions where several 5'-ends occur close together (see lanes *d* and *e* of Fig. 1). The pattern produced by exonuclease VII mapping is similar to the *S*₁ nuclease pattern but is more discrete. Comparison of the *S*₁ pattern and the direct 5'-end analysis data^{19,21} with other data in the literature²², shows that the major band of the *S*₁ nuclease-digested DNA is two nucleotides longer than its RNA counterpart. This phenomenon is even more pronounced with exonuclease VII where in most cases the DNA is four to five nucleotides longer than the RNA to which it was hybridized (possibly the enzyme is inhibited by

the positive charge on the cap structure). The patterns of the initiation points which can be deduced from the gel analyses reported here, are very similar to those obtained from reverse transcription mapping^{18,20}. Note also that some of the late 5' initiations start in the 72-bp repeats (Fig. 1).

To test the hypothesis that the remarkable heterogeneity of initiation points is caused by tandemly repeated promoter elements¹⁶, a series of deletion mutants was constructed by nuclease *Bal*31 digestion of *Sph*I-opened SV40 DNA cloned in plasmid pBGF (D.G. *et al.*, in preparation; see also Fig. 1). Analysis of the late mRNA obtained from CV1 cells infected with the largest viable mutants, dl 1845 and dl 1846, and with the natural deletion mutant, dl 894, is shown in Fig. 2. The analysis involved exonuclease VII digestion with a DNA probe labelled at the *Hind*II site at position 472 (similar results have also been obtained with an *Hpa*II probe labelled at position 348). Four major conclusions arose from these experiments: (1) The analyses showed that the initiation patterns downstream from the deleted sequences were almost unchanged when compared with the wild-type 5'-termini, suggesting that

Fig. 3 Mapping of the 5' ends of SV40-specific RNA synthesized in *X. laevis* oocyte nuclei injected with wt templates. Stage VI oocytes were injected with 10 ng sucrose gradient-purified DNA aiming for the nucleus^{23,49} and incubated at 22 °C for 6 h. The nucleus and cytoplasm were separated after heating the oocyte at 75 °C for 3 min, followed by making an incision and manual removal of the nucleus. The nuclear RNA was isolated by hot phenol extraction and the preparation was further digested with proteinase K-treated DNase I⁵⁰ (twice for 15 min at 37 °C with 1 µg per nucleus), phenolized with hot phenol and co-precipitated with the isolated [5'-³²P]-labelled single-stranded probe. Hybridization and exonuclease VII digestion were as described in Fig. 1 legend. *a*, RNA from uninjected oocytes; *b*, reference pattern of poly(A)⁺ mRNA from CV1-infected cells (same as lane *b* in Fig. 2); *c*–*e*, RNA from oocytes injected with: *c*, viral wt DNA; *d*, pSCLO₄ DNA; *e*, pSMboC DNA; *f*, not relevant to this experiment; *g*, undigested single-stranded probe. Each gel analysis was carried out with three oocyte RNA equivalents. The wt templates pSCLO₄ and pSMboC plasmids were constructed as follows: pSCLO₄ contains the SV40 *Hind*II+*Hind*III fragments C and L (568 nucleotides) and was made by addition of a *Bam* linker to the *Hpa*I site at position L501 and cloning between the *Hind*III and *Bam*HI sites of pBR322. pSMboC contains the SV40 *Mbo*II fragment C (710 nucleotides) which was blunt-ended, *Bam* linkers were added and the fragment was cloned in the *Bam*HI site of pBR322. The SV40 formation in both pSCLO₄ and pSMboC spans the region of DNA replication and transcription initiation.

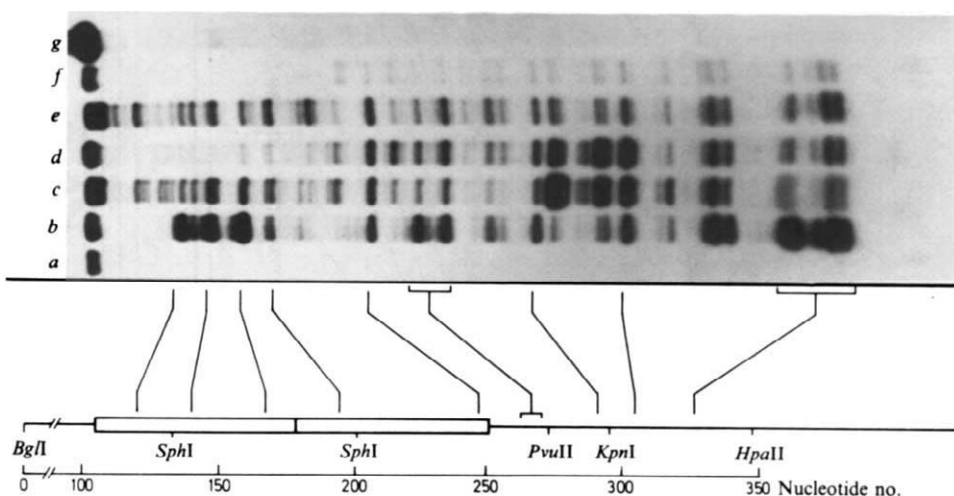
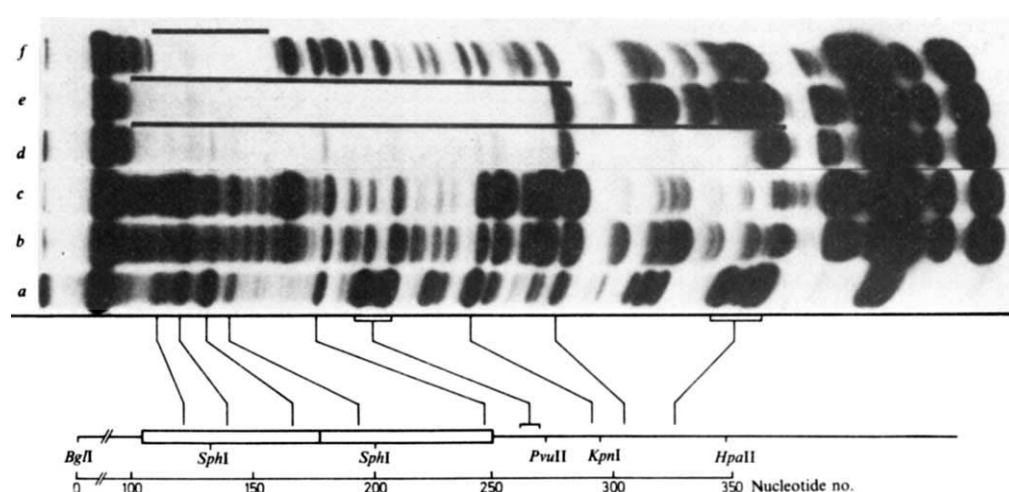


Fig. 4 5'-End analysis of oocyte nuclear RNA synthesized on SV40 wt and deletion mutant templates. The experimental procedure was as described in Fig. 3 legend. *a*, CV1 wt poly(A)⁺ mRNA reference pattern (same as for Fig. 2*b*); *b*, injection with wt viral DNA, 4 h incubation; *c*, injection with wt viral DNA, 22 h incubation; *d*, injection with mutant pS1849 DNA; *e*, injection with mutant pS1850 DNA; *f*, injection with mutant dl 1846 DNA. The position of the deletions is shown by a heavy horizontal line above the corresponding lanes (see also the map of the mutants at the bottom of Fig. 1). The darker band in lanes *d* and *e* near the *KpnI* site is a background band, as no initiation sites can be present at this position.



the deletions in the regions immediately upstream have little function in the positioning and synthesis level of the remaining RNA species. (2) As these viable deletions also delete major parts of the 72-bp 'enhancer' sequences, which are required for early gene expression², it seems that the most crucial elements of the 'enhancer' sequences are localized at the right part of the repeat and/or that other regulatory mechanisms (for example, T-antigen autorepression) back-up for a decreased enhancer function. (3) The dl 1846 pattern, the largest viable deletion in this region, shows a new strong band located at the end of the deletion, which could represent an artificial new initiation point formed by the combination of nucleotides around the deletion; alternatively, it could be derived from RNAs initiated in foreign DNA inserted at the position of the deletion. Indeed, it was found (D.G. *et al.*, in preparation), that the viral DNA collected after CV1 infection, especially that from the mutant dl 1846, showed some heterogeneity in the *Hind* C fragment (the mutant dl 1845 was found to be more stable in this respect). To avoid this complication, late SV40 transcription was also analysed in *X. laevis* oocytes (see below). (4) There is also a predominant initiation point around position 433. This site probably corresponds to the initiation sites mapped by Piatak *et al.*²⁰ at positions 428–481. Again, the position of this initiation point was not shifted by extensive upstream deletions.

During the construction of the deletion mutants, several non-viable mutants having more extensive deletions in the presumed promoter region were also isolated (for example, pS1849 and pS1850). The transcriptional behaviour of these and the other mutants was examined in *X. laevis* oocytes.

Initiation of wild-type and mutant late SV40 transcripts in nuclei of *X. laevis* oocytes

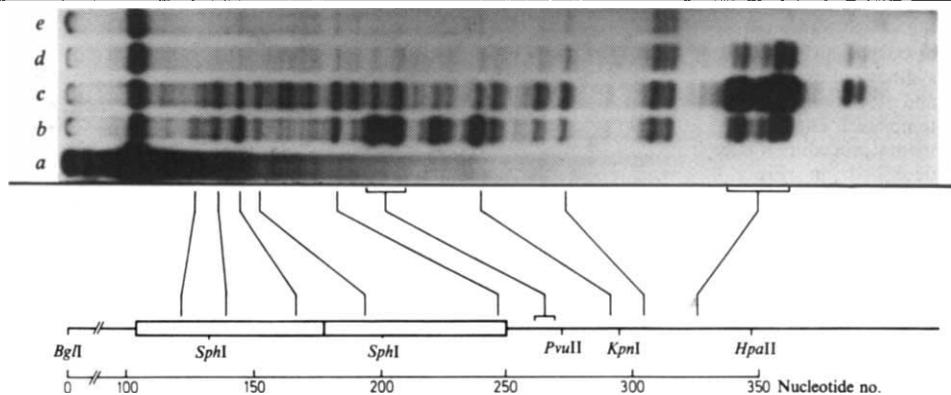
Xenopus oocytes have been shown to synthesize late^{23,24} and early²⁵ SV40-encoded products. Faithful promoter-specific initiation, however, has not yet been demonstrated. Figure 3 shows the 5'-end analysis of SV40-specific RNA synthesized in *X. laevis* oocytes injected with various wild-type templates (Fig. 3*c–e*) compared with *bona fide* late mRNA from CV1 cells (Fig. 3*b*). It is evident that the wild-type viral RNA initiation pattern in oocytes (Fig. 3*c*) closely resembles the pattern found in CV1 cells (Fig. 3*b*). There are some small differences, mainly quantitative, in the relative strength of transcription initiation, rather than qualitative differences in the pattern of transcription. At later times (22 h), these differences increase but the basic pattern remains the same (Fig. 4*c*). The results indicate that oocyte nuclei provide a reliable and faithful system for the study of SV40-directed transcription at least at

short times after injection (6–7 h). Injection with the SV40 fragments *Hind* C+L (position E67–L501; for numbering see Fig. 1 legend) or *Mbo*II C (position E236–L474) containing the expression control signals cloned in a pBR322 derivative, again produced the typical late transcription pattern (Fig. 3*d* and *e*, respectively). Therefore, it may be concluded that the fidelity of the transcription of the SV40 sequences is not appreciably affected by neighbouring plasmid sequences. In addition, it is clear from the results obtained with these plasmids that information for large T antigen is neither needed for efficient late transcription in oocytes nor involved in positioning of the late mRNA start sites. This situation resembles the *in vitro* transcription initiation pattern of early SV40 RNA¹⁵ which is similar to an *in vivo* late-early pattern, although it was found that late time initiation patterns of 'in vivo' early mRNA do require the presence of a functional T antigen²⁶. Injection of the non-viable mutant DNA, pS1850 and pS1849 (see Figs 1, 4), which lack almost the entire late mRNA initiation region, showed that here again the deleted sequences apparently have no effect on the downstream initiation points. Therefore, the results obtained with deletion mutants in CV1 cells described here and by others^{19,20}, and those obtained with extensive deletion mutants in injected oocytes, indicate that unlike the normal system of a classical promoter-driven transcription, the control elements for SV40 late transcription are not localized in the regions immediately upstream (or downstream) from the mRNA initiation site. As discussed above, various data in the literature suggest that DNA replication is linked to the activation of late transcription. To test the possibility of a direct involvement of the SV40 DNA replication region in late transcription, we injected the DNA replication-negative mutant 8-4^{17,27} into *X. laevis* oocytes.

Transcription of a DNA replication-negative mutant in *Xenopus* oocytes

The 5'-end of the late SV40-specific RNA synthesized in *X. laevis* oocytes injected with the plasmid pSCLO₄ (containing the SV40 control region from E67 to L501) and with the DNA replication-negative mutant 8-4 DNA, is shown in Fig. 5 (lanes *c* and *d*, respectively). Clearly the amount of late SV40 RNA isolated from the nucleus of mutant 8-4-injected oocytes is greatly reduced, although the remaining mRNAs are still faithfully initiated. The possibility of some artefact such as faulty nuclear injection with the mutant DNA is excluded by two different controls. First, in the experiments shown, wild-type and mutant DNAs were mixed with an equal amount of DNA from the plasmid p(325)-gHFIF-4, which contains the gene for human fibroblast interferon²⁸, and the incubation medium of the injected oocytes was assayed for interferon. The results show even better interferon synthesis by the oocytes injected

Fig. 5 5'-End analysis of SV40-specific late RNA synthesized in *X. laevis* oocyte nuclei injected with a plasmid containing an SV40 DNA replication-negative mutant. *a*, Undigested single-stranded probe; *b*, poly(A)⁺ mRNA of wt-infected CV1 cells, reference pattern; *c*, injection with the plasmid pSCLO₄ containing a functional wt SV40 DNA replication region (same as used in Fig. 3*d*) plus p(325)-gHFIF-4 DNA as an internal control; *d*, injection with the DNA replication-negative mutant 8-4 (see below) plus p(325)-gHFIF-4 DNA as an internal control; *e*, nuclear RNA from oocytes injected with plasmid p(325)-gHFIF-4, which encodes human fibroblast interferon²⁸ (internal control for lanes *c* and *d*). The mutant 8-4 was constructed by Gluzman *et al.*¹⁷ by directed mutagenesis of pMK16/SV40 DNA at the *Bgl*I site. The deletion of four nucleotides at the *Bgl*I site does not influence the synthesis of T antigen (and hence the major type of early mRNA synthesis), but prevents DNA replication and production of V antigen and infectious virus¹⁷.



with mutant than by those injected with wild-type DNA. The validity of the experiment was further corroborated by data obtained from the analyses of the early SV40 RNAs synthesized in the same oocyte nuclei (see Fig. 6), which showed that the amount of early RNA from the DNA replication-negative mutant is even higher than the amount of RNA from the wild-type-injected oocytes (the higher early transcription may be coincidental or may be due to the absence of competition for transcription factors by an inactive late region). Densitometer tracings of the gels and visual interpretation indicate that there is a reduction of at least 10-fold in late SV40 RNA synthesis by the DNA-replication-negative mutant compared with transcription from the wild-type template. The possibility of gene copy number amplification (despite the non-permissivity of this system²⁹) of the wild-type pSCLO₄ DNA was analysed by quantitation of the DNA by dot hybridization and by analysing the methylation pattern of the injected DNA. Although by dot hybridization we could not exclude a slight increase (1.3–1.9-fold) in the amount of nuclear plasmid DNA 7 h after injection, the methylation pattern of the DNA as analysed by a Southern blot of *Mbo*I and *Sau*3A digests was unchanged during the incubation, indicating the absence of even a single round of DNA replication (data not shown). In

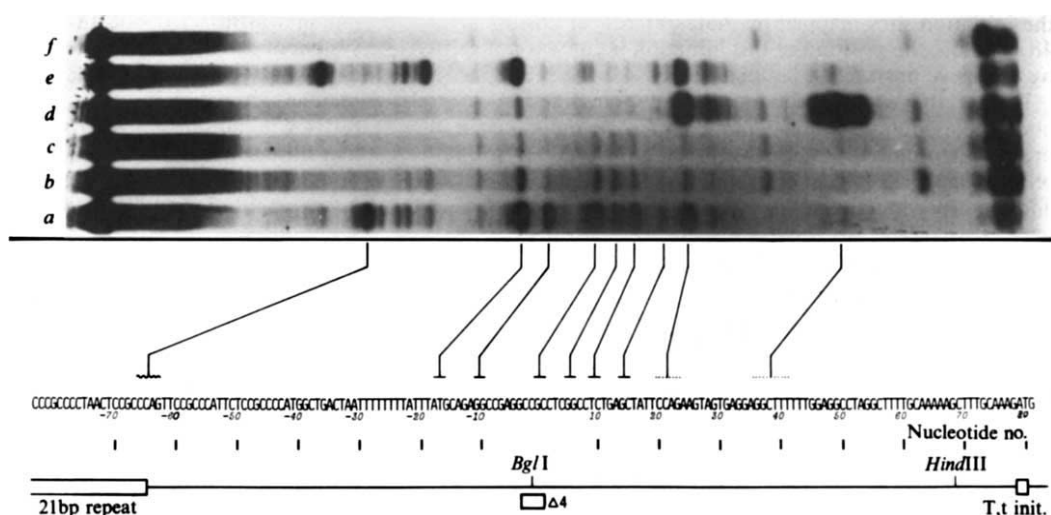
addition to the quantitative aspect of late transcription by the origin-defective mutant, there is also a difference in the initiation pattern of early transcription. It was observed that the initiation positions of early RNA which appear only at late times after infection^{26,30} (after the onset of DNA replication) are used considerably less by the origin-defective mutant than by the wild-type DNA (Fig. 6*b, d*). Instead, the more rightward initiation points around nucleotide E40 are more pronounced. We propose that in addition to the classical polymerase II promoter for early transcription, there are heterogeneous early transcripts which are formed as a result of the activation of the DNA replication origin.

Discussion

RNA polymerase II promoters have been well characterized in different (*in vitro* and *in vivo*) systems by the construction of deletion mutants in critical regions. In this manner the function of the TATA box was elucidated^{31–33}, evidence for the function of a –70 signal (the CAAT box) was obtained from studies *in vivo* (including oocytes)^{34–36}, and a more upstream ‘enhancing’ function was found^{2,37}.

The late transcription unit of SV40 is unlike most cellular or viral transcription systems because it is only actively expressed

Fig. 6 5'-End analysis of SV40-specific early RNA synthesized in *X. laevis* nuclei. *a*, Poly(A)⁺ mRNA from wt-infected CV1 cells, reference pattern (48 h after infection); *b, c*, injection with plasmid pSCLO₄ containing a wt DNA replication region (same RNA as used in Fig. 5*c* for analysis of late SV40-specific RNA); *d*, injection with the plasmid containing the SV40 DNA replication-negative mutant 8-4 (same RNA as used in Fig. 5*d* for analysis of late SV40-specific RNA); *e*, injection with pS1846 DNA (same RNA as used in Fig. 4*f*); *f*, the probe incubated in the presence of poly(A)⁺ mRNA from uninfected CV1 cells. The dark full-size band near the top of the gel is due to contamination of the [5'-³²P]HindIII-labelled single-stranded probe with some complementary strand and renaturation. At the bottom, note the nucleotide sequence (from 5' to 3') of the strand having the same polarity as the early SV40 mRNA (unlike the diagrams below Fig. 1–5 which dealt with late transcription). The early transcription is from left to right. The initiation points were identified relative to a chemical degradation pattern⁴⁶ run on another gel. The RNA initiation points do not correspond exactly with those given by Ghosh and Lebowitz²⁶, but the differences may be due to the different methods used and these precise assignments do not affect our main conclusions. The leftmost initiation point is mapped less accurately (wavy line). Also indicated on the diagram is one of the 21-bp repeats, the 4-nucleotide deletion at the *Bgl*I site in the mutant 8-4, and the start codon for synthesis of small t- and large T-antigen synthesis. (T, t init.).



in permissive cells, and because of its high degree of transcriptional start site heterogeneity. Neither our own results for CV1 cells described here, nor the results of others with SV40 (ref. 38 and W. Schaffner, personal communication) or with polyoma virus³⁹, have indicated a function of the aforementioned classical promoter elements for late viral transcription. Therefore, an analysis of larger (and hence non-viable) deletions in the region upstream of the major transcription initiations was required. We therefore explored the possibility of studying the late SV40 transcription system in nuclei of *Xenopus* oocytes and found that it was faithful. As in the CV1 system, however, we were unable to find any evidence of classical polymerase II promoter elements by analysis of a series of appropriate deletion mutants. Instead, we found a clear coincidence between the DNA replication function and an essential function required for late RNA synthesis located within four nucleotides at the *Bgl*I site. This unexpected result can be explained by assuming either that the region of DNA replication is directly required for late mRNA synthesis, or that the overlapping of functions is purely incidental. The latter explanation would imply the presence of a remote control element, as yet unidentified, that would be involved in transcriptional initiation at sites some of which are over 300 nucleotides downstream. We therefore consider it more likely that the mutation 8-4 at the *Bgl*I site¹⁷ largely abolishes late transcription due to an impaired DNA replication function.

The 'special' structure present in SV40 DNA, which has an active origin of DNA replication and which functions as a novel type of polymerase II promoter, may be either a 'DNA replication eye', a D-loop structure, a region where special DNA replication proteins bind, or a special conformation of the origin DNA formed in the nuclear environment. This 'special' structure is presumed not to be formed or to be formed to a considerably lesser extent by the *Bgl*I deletion mutant, 8-4.

Hay and De Pamphilis⁴⁰ recently proposed that SV40 DNA replication starts with the synthesis of DNA having the same polarity as early mRNA, whereby a single-stranded region is exposed on the retrograde template which is the same strand as is transcribed for late RNA synthesis. As it was generally found that class II RNA polymerases prefer denatured DNA as template⁴¹, this single-stranded region on the retrograde arm may allow the formation of a stable complex with the RNA polymerase which then begins to travel along the template and initiates RNA synthesis at many but non-random positions, giving rise to late SV40 mRNA transcripts.

It may be argued that *X. laevis* nuclei do not constitute a permissive system for SV40 DNA replication but as mentioned

above, the special structure formed by the origin of DNA replication and implicated in late SV40 transcription does not necessarily even involve incipient DNA strand synthesis. It may be formed either by properties intrinsic to the DNA sequence itself or by interaction with nuclear components whose properties are conserved even between CV1 cells and *X. laevis* oocytes. In permissive monkey cells, T antigen may be responsible for the progression of the special structure to an active DNA replication complex. In non-permissive cells, such as mouse or rat cells, T antigen which binds to the replication origin region¹⁴ may be responsible for shut-off of all late transcription. Of course, additional factors may be involved in distinguishing a permissive system from a non-permissive system (for example, factors needed for expression of the V antigens). Note, however, that Subramani *et al.*⁴² have observed expression in non-permissive cells of a dihydrofolate reductase gene cloned in the late region of SV40.

Ghosh and Lebowitz²⁶ have shown that there is an increase in 5'-terminal heterogeneity of early SV40 mRNA at late times in infection. Similar observations had been reported for polyoma virus early mRNA³⁰. If the heterogeneity of the late RNA initiations were coupled to the formation of a special DNA structure in the origin region, as we propose, then the increase in heterogeneity of early start points could be produced by a similar mechanism—that is, direct activation of a second type of transcription system by the DNA replication region.

Thus, we believe we have demonstrated a novel type of gene regulation. RNA polymerase II can either initiate transcription on classical promoters or it can initiate by a mechanism driven by a DNA replication origin. Considering that many genes are regulated in function of the cell cycle, it certainly seems meaningful to couple the expression of several genes to DNA replication. Indeed, the phenomenon of regulation of late SV40 gene expression by DNA replication may also have a role in the expression of some cellular genes, such as the regulation of histone synthesis as observed in yeast⁴³, Chinese hamster ovary cells⁴⁰ and hamster fibroblast cells⁴⁴.

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LETTERS TO NATURE

Are there black holes in quasars?

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There are two main theoretical models for the central engine of quasars and other active galactic nuclei: the black hole model and the spinar model. Observations of the time scales of flux variations can rule out the black hole model and are therefore extremely important. The widely accepted argument of Elliot and Shapiro¹ states that the maximal luminosity of the nucleus is given by the Eddington limit, $L_{\text{Edd}} = 10^{38} (M/M_{\odot}) \text{ erg s}^{-1}$, and the minimal time scale for periodicity is given by $t_{\text{min}} = (\text{gravitational radius})/(\text{velocity of light}) = 10^{-5} (M/M_{\odot}) \text{ s}$. Therefore, the observed time scale of variations t , and the luminosity L must obey $\log t > \log L - 43$. Variations on a time scale shorter than that would rule out the black hole model. We point out here that this statement is wrong in view of recent developments in the accretion disk theory: much shorter periodicities are still consistent with the black hole model.

One of the most important new results of the theory of accretion disks is that the observed flux of geometrically thick disks can be formally consistent with luminosities up to 100 times (but probably not much more) greater than the Eddington luminosity²⁻⁵. This is not only due to genuine super-Eddington luminosities of the disks, but mainly because the radiation is highly beamed in a long and narrow vortex along the axis of

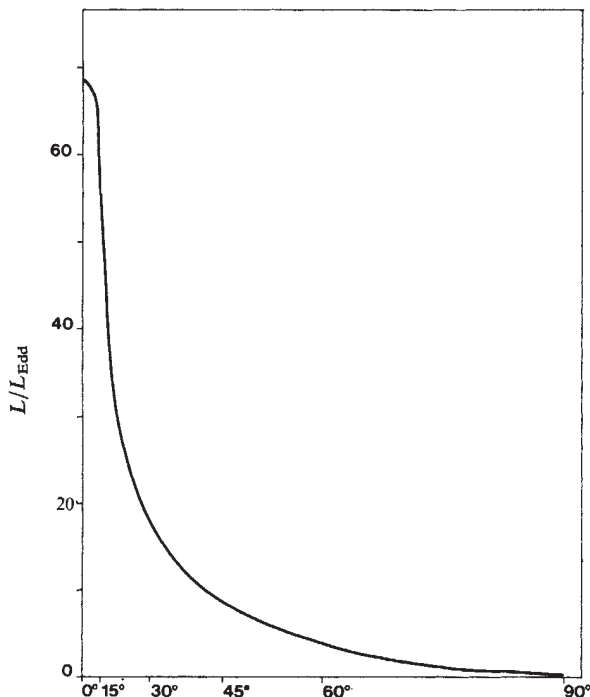


Fig. 1 Beam of radiation emerging from the funnel of a thick accretion disk. The luminosity L formally obtained from the observed flux (by assuming an isotropic radiation field) depends on the angle θ which line of sight makes with the axis of the beam. Luminosities substantially greater than L_{Edd} are possible.

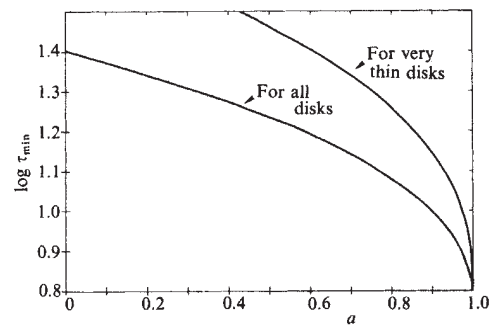


Fig. 2 $\log \tau_{\text{min}}$ plotted against the Kerr parameter a . Although the condition $\log \tau_{\text{max}} > 0.8$ cannot distinguish between thin and thick disks, one must remember that if $\lambda \gg 1$, the thin disks are ruled out.

rotation. This high flux in the beam, when multiplied by the surface of the whole celestial sphere, can give formally a luminosity of the order of $100L_{\text{Edd}}$, when the intrinsic luminosity of the nuclei is only a few Eddington luminosities. The possibility that the radiation of quasars and other active galactic nuclei, especially BL objects, is highly beamed was suggested a few years ago by Blandford and Rees⁶. The agreement of this hypothesis with quasar statistics is very good⁷. Figure 1 (taken from ref. 5) shows how highly the radiation is beamed. The observed flux is formally consistent with a luminosity

$$L = \lambda L_{\text{Edd}} = 1.26 \times 10^{38} \lambda \left(\frac{M}{M_{\odot}} \right) \text{ erg s}^{-1} \quad (1)$$

where the dimensionless, model-dependent, parameter λ obeys the condition $\lambda \leq 10^2$.

Another important recent result of the theory of accretion disks is that the innermost edge of the disk does not necessarily coincide with the marginally stable orbit, $r = r_{\text{ms}}$, but can be much closer to the hole: as close, in fact, as the marginally bound orbit, $r = r_{\text{mb}}$. Frequency of circular orbital motion gives a good estimate of a minimal possible periodicity of flux generated at a given point on the surface of the disk⁸

$$t_{\text{min}} = \tau \frac{r_{\text{G}}}{c} = 0.98 \times 10^{-5} \tau \left(\frac{M}{M_{\odot}} \right) \text{ s} \quad (2)$$

with the dimensionless parameter $\tau \equiv \pi(r_*^{3/3} + a)$ depending on the location of the region which provides the time variation, $r = 1.48 \times 10^5 r_*(M/M_{\odot}) \text{ cm}$, and on the dimensionless Kerr parameter a which characterizes the rotation of the black hole: when $a = 0$ the hole does not rotate, and when $a = 1$ the hole rotates at the maximum possible rate. The locations of the horizon, marginally bound, and marginally stable orbits for the non-rotating black hole are given by $r_* = 2$, $r_* = 4$, and $r_* = 6$ respectively; for the hole rotating at the maximum speed all

Table 1 Estimated values of a_{min} for seven observational points shown in Fig. 3

Ref.	Type	Object	z	log L	log t	a_{min}		
						$\lambda = 10$	$\lambda = 30$	$\lambda = 100$
1	Q	3C454.3	0.860	46.1	3.0	0.90	0	0
1	Q	3C345	0.594	46.2	3.4	0.33	0	0
1	BL	BL Lac	0.07	45.5	2.3	0.98	0	0
9	BL	1308	0.996	47.5	4.5	0.78	0	0
9	BL	0235	0.582	48.2	4.7	*	0.78	0
9	Q	3C446	1.403	48.1	4.6	*	0.78	0
10	BL	OJ287	0.306	47.0	2.97	*	*	0.78

BL, BL Lacerte; Q, quasar; * black hole model ruled out.

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three locations are given by $r_* = 1$. Therefore, the parameter τ must obey $\tau > \tau_{\min} = 2\pi$, or $\log \tau_{\min} = 0.8$ for an $a = 1$ hole and $\tau > \tau_{\min} = 8\pi$ or $\log \tau_{\min} = 1.4$ for an $a = 0$ hole.

The function $\tau_{\min}(a) = \pi[(r_*)_{\text{mb}}^{3/2} + a] = \pi\{[2 - a + a(1 - a)^{1/2}]^{3/2} + a\}$ is shown in Fig. 2.

From equations (1) and (2) we deduce the corrected form of the basic Elliot-Shapiro formula

$$\log t_{\min} = \log L - 43.1 + \log(\tau/\lambda) \quad (3)$$

If the source of radiation is undergoing relativistic motion then, as pointed out by Blandford and Rees⁶, the observed time variation can be even shorter. This, however, cannot be applied to periodic variations observed over many periods as these must be connected with disk rotation. Knowing from observation the values of $t_{\min}$ and L one can find from equation (3) the value of $\log(\tau/\lambda)_{\text{obs}}$. Assuming that $\lambda = 10^2$ is an upper limit for luminosity one can find the upper limit for $\log \tau$, equal to $\log \tau_{\max} = \log(\tau/\lambda)_{\text{obs}} + 2$. If $\log \tau_{\max} < 0.8$ then one can say that observations exclude the possibility of the black hole model. In the case when $0.8 < \log \tau_{\max} < 1.4$ non-rotating black holes are ruled out. Observations are still consistent with rotating black holes for which the Kerr parameter a is bigger than the minimal one, $a_{\min}$, given by the solution of the equation

$$a_{\min}: \log \tau_{\max} = \log \tau_{\min}(a) \quad (4)$$

Figure 3 shows observational points taken from refs 1, 9 and 10 for seven objects which are closest to the original Elliot-Shapiro restriction (heavy line). The observations of OJ287 by Valtaoja *et al.* which suggest the most extremal flux and the most rapid rotation of the black hole are very recent ones and must still be confirmed. The five thin lines give the shortest possible $t_{\min}$ (that is for $\log \tau = 0.8$) in the case when $\lambda = 1, 3, 10, 30$ and $\lambda = 100$. In Table 1 we present estimations of $a_{\min}$ for these seven points. All the observational points are consistent with the black hole model. Figure 3 indicates that thick, but not thin, accretion disks can explain observations. In some cases observations show that the central black hole must rotate. More detailed analysis of the greater body of observational data is now in progress and the results will be published elsewhere.

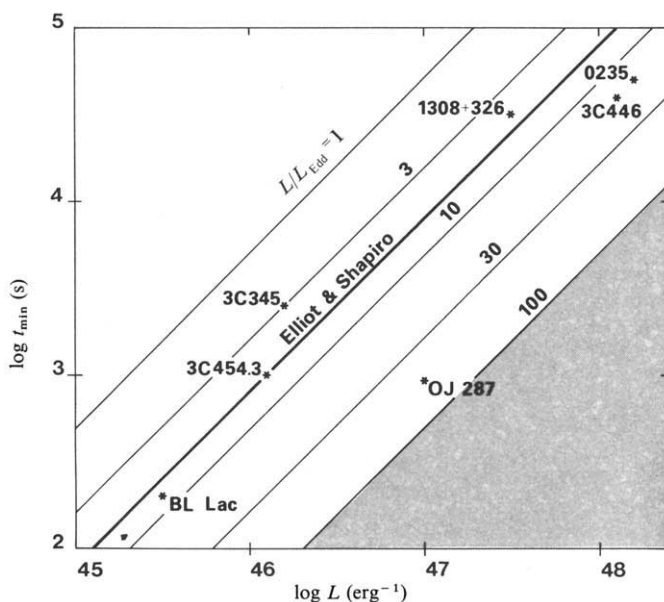


Fig. 3 Seven active galactic nuclei on the $\log L$ versus $\log t$ plane. The heavy line corresponds to the original Elliot-Shapiro relation ($\lambda = 1 = \tau$). Three thin lines give absolute lower limits for $t_{\min}$ in the cases $\lambda = 1, 3, 10, 30, 100$ ($\log \tau = 0.8$ is assumed on these lines). For almost all the objects $\lambda \gg 1$. This rules out thin accretion disks. Observational points in the dark region would contradict the black hole model.

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$\text{H}_2\text{CN}^+ \cdot n\text{N}_2$ clustering formation and the atmosphere of Titan

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Measurements performed by Voyager 1 have revealed a dense atmosphere around Titan mainly composed of N_2 with some CH_4 and other minor components, including hydrocarbons and organic-nitrogen molecules¹⁻⁴. Based on Voyager and some laboratory data, it is reasonable to assume that other complex organic-nitrogen molecules are formed in Titan's atmosphere⁵⁻⁷. Three-body association reactions are probably involved in the synthesis of such compounds^{5,7,8}. It has been suggested⁵ that the clustering of H_2CN^+ with N_2 or CH_4 could have an important role in these reaction mechanisms. Recently,⁸ we observed the $\text{H}_2\text{CN}^+ \cdot \text{N}_2$ cluster ion in a N_2 - CH_4 mixture irradiated by α particles. Here we report the results of laboratory measurements of equilibrium constants for the clustering of N_2 with H_2CN^+ leading to $\text{H}_2\text{CN}^+ \cdot n\text{N}_2$ ($n = 1-6$) formation, which should help in understanding the ion-molecule chemistry in the atmosphere of Titan.

The chemical complexity of Titan's atmosphere detected in the course of the Voyager 1 encounter has been accepted to be a result of the coupled chemistry of ion-molecule and neutral reactions^{4,5,7,9}. In the lower atmosphere of Titan, where ionization results from cosmic-ray bombardment, lower temperatures and higher neutral densities are suitable conditions for three-body ion-molecule association reactions. The temperature and pressure in the region of maximum cosmic-ray activated chemistry are $\sim 150-160$ K and ~ 20 mbar (ref. 5).

The ion H_2CN^+ probably has a major role in the chemistry of Titan's atmosphere: it can be associated with N_2 , CH_4 and C_2H_2 molecules leading possibly to formation of compounds such as CH_3CN , $\text{C}_2\text{H}_3\text{CN}$... through three-body ion-molecule reactions, while it is assumed to be partially responsible (with $\text{N}(^4\text{S})$ and $\text{N}(^2\text{D})$) for HCN formation¹⁰. Although Voyager 1 did not directly observe any evidence of the presence of the H_2CN^+ ion, electron and ion measurements¹¹ suggest the presence of an ion with a mass of the order of 28 AMU, which is believed¹¹ to be H_2CN^+ . Recently¹², this ion has been tentatively identified in the Earth's stratosphere: in fact, some of the chemical processes involved in the formation of prebiotic molecules in the atmosphere of Titan are probably similar to those which could have acted in the primitive Earth's atmosphere.

To gain some insight into the ion chemistry on Titan, we carried out a simulation experiment⁸ in temperature, pressure and atmosphere composition conditions consistent with the interpretation of the Voyager 1 data. In this, a gas mixture of

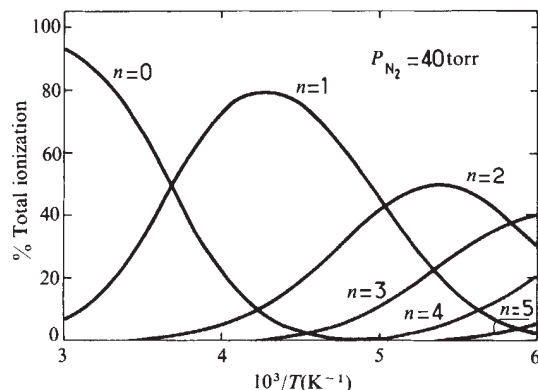


Fig. 3 Equilibrium distribution of ions $\text{H}_2\text{CN}^+ \cdot n \text{N}_2$ predicted by experimental data.

dominated by physical bonding (polarizabilities). There are some common features between the present results and those obtained by Hiraoka; for example (1) spacing between the lines of the van't Hoff plots shows a larger gap between the (0, 1) and (1, 2) equilibria; (2) the $\Delta H_{n-1,n}^\circ$ values are found to be almost constant between (2, 3) and (4, 5); (3) these values are found to be nearly the same between (2, 3) and (4, 5) equilibria. The first feature indicates that $\text{H}_2\text{CN}^+ \cdot \text{H}_2$ and $\text{N}_2\text{H}^+ \cdot \text{N}_2$ are much more stable (towards loss of N_2) than the higher n complexes; the second feature shows that the stability decreases in that range are almost entirely due to entropy decreases. Finally, in both cases, $\Delta H_{n-1,n}^\circ$ decreases as n increases ending ultimately to the heat of vaporization of liquid N_2 ($\sim 1.3 \text{ kcal mol}^{-1}$).

The present results are consistent with the statement made by Capone *et al.*⁴ on the possibility of $\text{H}_2\text{CN}^+ \cdot \text{N}_2$ association by three-body ion clustering reactions in the atmosphere of Titan. Furthermore they show that higher order associations are not excluded in this atmosphere.

Nevertheless, the high-altitude detection by Hartle *et al.*¹¹ of an ion of mass of the order of 28 AMU could also be interpreted as C_2H_5^+ (29 AMU) or N_2^+ (28 AMU). These ions are effectively observed in our mass spectra, as well as $\text{C}_2\text{H}_5^+ \cdot (\text{N}_2)_{1-5}$, $\text{CH}_5^+ \cdot (\text{N}_2)_{1,2}$ and $\text{CH}_3^+ \cdot (\text{N}_2)_{1,2}$. The lower limits for enthalpy and entropy changes for the three-body association reactions leading to $\text{C}_2\text{D}_5^+ \cdot (\text{N}_2)_{1,2}$ formation have been determined¹⁴ recently in N_2 - CD_4 mixtures. Moreover, the presence of small amounts of C_2H_2 in the atmosphere of Titan must have a very important effect on the ionic populations as we observe¹⁵ in this experiment: ions such as $[(\text{C}_2\text{H}_2)_n(\text{N}_2)_m]^+$ and $\text{X}^+(\text{C}_2\text{H}_2)$ (where $\text{X} = \text{C}_3\text{H}_3, \text{C}_3\text{H}_4, \text{C}_3\text{H}_5, \dots$) have been detected with very small admixture of C_2H_2 in N_2 and N_2 - CH_4 mixtures respectively.

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Enhanced soft X-ray detection efficiencies for imaging microchannel plate detectors

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Position-sensitive detectors using microchannel plate (MCP) electron multipliers have been widely used in X-ray astronomy¹⁻³. Although MCPs facilitate X-ray imaging with high spatial resolution, as on the Einstein¹ or EXOSAT² satellites, their intrinsic soft X-ray detection efficiencies are low. These lie typically in the range 1-10% (ref. 4), much lower than the (near unity) values attainable with competing forms of gas proportional counter. To enhance sensitivity to X-ray energies below a few keV, a material of relatively high photoelectric yield may be deposited on the MCP front surface and channel walls. MgF_2 has commonly fulfilled this role for X-ray astronomy¹⁻³, resulting in enhanced efficiencies 1.1-1.6 times those of uncoated MCPs¹. We report here high 0.18-1.5 keV X-ray detection efficiencies for MCPs bearing CsI deposition photocathodes. Efficiency enhancement factors, dependent on X-ray incident angle, of up to 15 times have been stably obtained. The importance of this work for the sensitivity of future X-ray astronomy experiments, such as the Röntgensatellit (ROSAT) Wide Field Camera, is outlined.

A microchannel plate, formed by the drawing and etching of a lead glass matrix, consists of many ($\sim 10^6$) hollow channels of common diameter 10-50 μm electrically connected in parallel. When a large bias voltage is applied along their length these channels can be made to act as a regular array of miniature photomultipliers. The many applications of MCPs have been reviewed by Wiza⁵. In the MCP's role as focal plane detector for grazing incidence X-ray optics^{1,3} its above-mentioned inefficiency, even with the small enhancements provided by MgF_2 coating, has been a major factor in determining overall X-ray telescope sensitivities.

The adoption of MgF_2 as a 'standard' MCP coating for X-ray astronomy has been based on its relative photoelectric stability in a normal laboratory atmosphere⁶ and not on its 'optimum' photoelectric yield properties in any absolute sense. A detailed study of X-ray photocathodes was therefore undertaken to identify, without experimental precondition, truly optimum candidate coating materials⁷. A collation of 1-300 Å X-ray photoemission data from many sources within a simple, predictive theoretical framework is given in ref. 7. CsI is found to be potentially a much superior MCP coating material to MgF_2 provided its hygroscopic nature—and hence possible instability in normal atmospheric humidity—can be accommodated by careful detector preparation and handling techniques.

Measurements were made on two position-sensitive X-ray detectors incorporating Mullard microchannel plates. The first of these (detector A) used a pair of MCPs of channel diameter $D = 12.5 \mu\text{m}$ in a 'chevron'⁸ arrangement. The input MCP (on which the CsI was deposited) had channels with a length-to-diameter ratio $L/D = 120:1$ biased at an angle $\phi = 4^\circ$ to the front surface normal. The rear plate of this chevron was characterized by $L/D = 80:1$, $\phi = 13^\circ$. The second detector (detector B) employed two 25- μm channel diameter MCPs, both of $L/D = 80:1$, with bias angles $\phi = 0^\circ$ (input MCP) and $\phi = 13^\circ$ (rear MCP).

The final stage in MCP manufacture is a reduction of the lead oxide glass content in a hydrogen atmosphere. New MCPs therefore copiously outgas water vapour and must be subjected to a prolonged vacuum bake before receiving a hygroscopic

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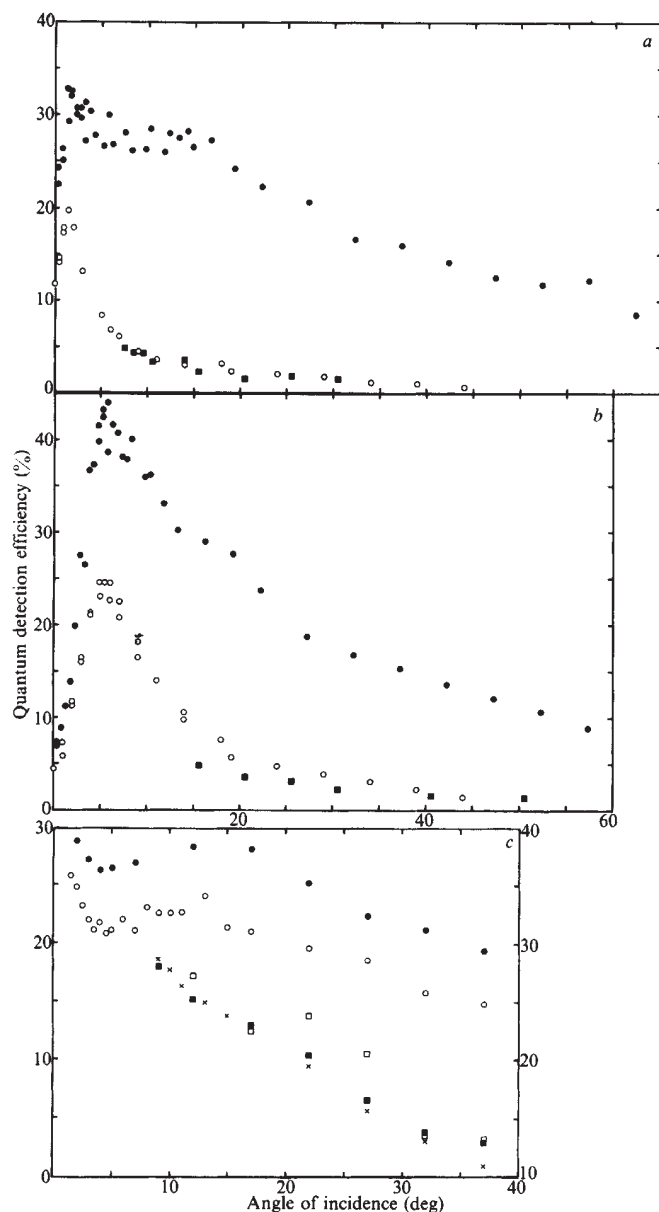


Fig. 1 Microchannel plate quantum detection efficiency as a function of X-ray incident angle. *a*, Al K X-rays, energy 1.5 keV. *b*, C K X rays, energy 0.28 keV. ●, Measurements on CsI-coated half of detector B. ○, Uncoated half of detector B. ■, Measurements on a MgF₂-coated MCP. *c*, Measurements on the CsI-coated half of detector A with Mg K (●) and Al K X-rays (○) (left-hand scale) and O K (■), C K (×) and B K (□) X-rays (right-hand scale). (For X-ray energies see text.)

coating. All our MCPs were vacuum-baked for 48 h at a temperature of 275 °C. The partial pressure of evolved H₂O was thus reduced by a factor of $\sim 10^3$, giving a final value of $\sim 10^{-10}$ torr (at room temperature) comparable with the partial pressures of secondary products—CO, N₂ and CO₂—revealed by mass spectral analysis.

CsI coatings were vacuum-evaporated onto one-half of each input MCP to a thickness of 6,000 Å on the MCP front surface. A nominal coating angle (between the molybdenum evaporation vessel and the normal to the centre of the MCP) of 15° was used for both detectors. The MCPs, preheated to 100 °C before CsI deposition, were rotated during the evaporation to ensure coating uniformity on the channel walls. The coating angle-front surface thickness combination used here produced a coating extending $\sim 3.7D$ down the channels with a characteristic thickness of 400 Å, about twice the escape length for X-ray-

induced secondary electrons in CsI (ref. 7). The coated MCPs were then assembled into their respective detector bodies. All manipulations and transfers of the MCPs after bake-out took place in a local atmosphere of dry N₂ with the coated plates protected additionally by removable detector caps whenever possible. Thus, on entry into the vacuum chamber used for efficiency measurements, the CsI coatings had been exposed to air for only a few tens of seconds.

A comprehensive study of detector gain and noise count was made for each detector at operating pressures between 1 and 2×10^{-5} torr. Satisfactory peak gains and narrow pulse height distributions were obtained for both detectors. A peak gain $G = 3 \times 10^7$ and FWHM $\Delta G/G = 45\%$ were easily achievable with detector A. Noise count rates of the order of $0.5 \text{ cm}^{-2} \text{ s}^{-1}$ ($3\text{--}5 \text{ s}^{-1}$ over total detector areas) were consistently obtained.

X-ray quantum detection efficiencies were measured for a range of X-ray energies and incident angles (the angle between the X-ray beam and the input MCP channel axis). The experimental technique was essentially that of Bjorkholm *et al.*⁸. The intensity of the incident X-ray beam, produced by a filtered, collimated fluorescence source, was measured using a pill-box type Ar-CH₄ (90:10 mixture) gas proportional counter⁶ of known window absorption. Figure 1*a* displays detection efficiency versus incident angle for both CsI-coated and bare glass halves of detector B illuminated by Al K X rays of energy 1.5 keV. Use of a CsI deposition photocathode has dramatically increased the MCP efficiency at all angles. The prompt nature of the photoemission process (on time scales of 10^{-14} s) and the long dead time of an individual microchannel ($\sim 10^{-2}$ s)⁵ dictate that the absorption of an X ray can give rise to at most one output pulse: the enhancement shown in Fig. 1*a* is not due to multiple pulses generated by a single photon. Figure 1*a* also shows measurements made in this laboratory on an MgF₂-coated MCP (M. Lewis, personal communication). The coating angles and thicknesses of the CsI and MgF₂ photocathodes were identical, although the pre-coating histories and quantum efficiencies of their MCP substrates differed. Figure 1*b* is the analogous figure for C K X rays, of energy 0.28 keV. All the efficiency-angle curves can be simply interpreted in terms⁴ of (1) the increase in photoelectric yield with decreasing grazing angle to the channel walls until (2) the onset of X-ray reflection, (3) the fraction of X rays incident on the coated channel walls, (4) the geometric transparency of the MCP, (5) the divergence of the test beam ($\pm 0.5^\circ$ FWHM).

Figure 1*c* shows efficiencies measured on the CsI-coated half of detector A for Al K, Mg K (1.25 keV), O K (0.525 keV), C K and B K (0.18 keV) X rays. Some spectral impurity of the O K line, arising from carbon contamination of the source anode, was possible with the present source-filter arrangement. Small-angle efficiencies have been omitted for clarity. Measured peak efficiencies in the order of decreasing X-ray energy were: 34.1, 35.6, 41.9, 48.1 and 38.3%. Detector A and B efficiencies are comparable, given the small differences between detectors due to bias voltage and channel geometry.

No measurements of the uniformity of the detection efficiency across the MCP surface were possible within the present vacuum system. A careful study of the photocathode deposition geometry has, however, been made to ensure that variations in the CsI coating profile were minimized within and between channels.

Repeat measurements on detector A were done to establish the ability of the CsI coating to withstand the vacuum breaking inevitable in the development of a flight detector for X-ray astronomy. No significant changes in gain, noise count or efficiency were noted during 11 cycles between vacuum and a dry N₂ environment, involving an accumulated input to the CsI of 5×10^5 photons mm^{-2} over a period of 18 days under continuously pumped vacuum. Detector B, by contrast, was subjected to several deliberate exposures to laboratory air. Relative humidity on exposure varied between 42 and 53% with ambient temperatures of 22–25 °C. No degradation in CsI efficiency was observed even after an accumulated exposure time of 8 h. Work

continues to establish the long term stability of the response of CsI-coated MCPs under various methods of storage. We note here that planar CsI XUV photocathodes have been found to be stable after 9 months of storage in dry N₂ (ref. 9).

These preliminary measurements indicate that CsI deposition photocathodes can provide a simple, stable and reproducible means of producing high efficiency microchannel plate X-ray detectors both for X-ray astronomy and other imaging applications⁵. We stress that the efficiencies reported here do not represent the limits attainable using a deposition photocathode technique. A thicker CsI layer, deposited at smaller coating angle, should appreciably increase peak efficiencies. Further, in the work discussed above, there is no contribution from the inter-channel area (37% of the MCP front surface) to the detection efficiency because of its large negative potential (~ -3.5 kV). As the input MCP is preceded by an earthed carbon-coated plastic film, photoelectrons released from the front surface are directed away from the channel entrances⁴. The use of a high transparency grid to establish an appropriate electron collecting field at the MCP input may be expected to increase detection efficiencies at all angles of incidence.

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Calculations based on the work described in refs 4 and 7 and supported by the present data, indicate that an MCP efficiency of 45% should be attainable for an X-ray energy of 0.1 keV and an incident angle of 30°. Such a combination is directly relevant to the Wide Field Camera (WFC), an XUV telescope now being developed by a consortium of UK research groups for flight on the German national ROSAT¹⁰ in 1987. The WFC is intended to carry out the first XUV (0.04–0.2 keV) sky survey. Use of a CsI-coated MCP is expected to increase the WFC sensitivity by a factor ~ 4 times relative to the MgF₂-coated detector of the design study (R. Willingale, personal communication).

Calculations also indicate an efficiency of at least 20% for 6-keV X rays incident at 4° to the channel axes, about six times the corresponding efficiency of an uncoated MCP. This energy-angle combination is of interest for focal plane instrumentation for AXAF¹¹, NASA's principal mission in X-ray astronomy planned for the next decade.

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Seasonal effects of temperature and salinity on the partial pressure of CO₂ in seawater

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The exchange of CO₂ between the atmosphere and the sea is of major importance to our understanding of the climatic consequences of anthropogenic CO₂. Because the solubility of CO₂ and the dissociation of carbonic acid in seawater are modulated by temperature and salinity, the detection of long-term changes in the oceanic carbonate system, first requires that the comparatively large short-term variations in the partial pressure of CO₂ should be characterized. We present here the first direct observations of seasonal variations in the partial pressure of CO₂ in the subtropical gyres of the North and South Pacific Ocean. These variations compare well with the variations predicted from observed changes in temperature and salinity using empirical equations which do not require the determination of alkalinity or total inorganic carbon, and the agreement is improved by the inclusion of air-sea exchange in the model calculations. Thus we predict that specific alkalinity within the two gyres remains extremely constant, which is confirmed by carbonate equilibrium calculations based on our measurements of total inorganic carbon.

The measurements were carried out as part of the NORPAX Equatorial Experiment during a series of month-long oceanographic expedition legs extending between Papeete, Tahiti and Honolulu, Hawaii. Continuous shipboard measurements of CO₂ partial pressures in surface waters were carried out between August 1979 and June 1980 aboard RV *Wecoma* on the north-bound expedition Legs 7, 9, 13 and 15. The analyses were made using automated dual-catalyst flame ionization gas chromatography¹ to determine the mole fraction of CO₂ in a dried aliquot of a gas phase which was equilibrated continuously

with seawater pumped from a depth of ~ 3 m. The results were corrected for slight warming in the pumping and equilibration system using the empirical temperature dependence given below (equation (1)). Measurements of atmospheric CO₂ mole fractions were also made by the shipboard chromatographic system. Standard deviation of replicate chromatographic CO₂ measurements is $\sim 0.04\%$, and the estimated systematic uncertainty of these measurements, including uncertainties associated

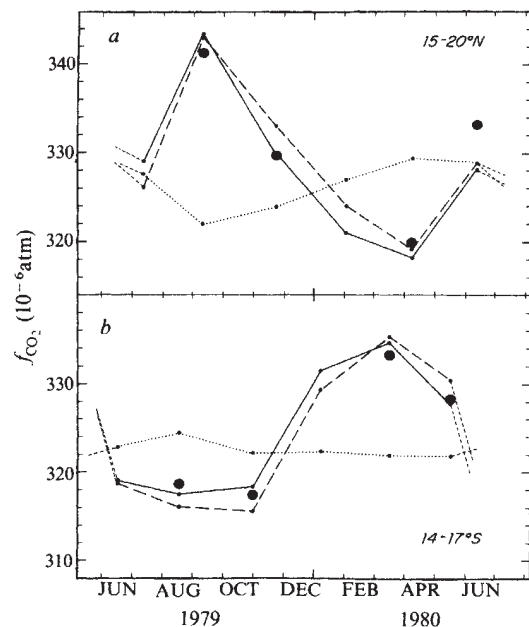


Fig. 1 Seasonal variations in the equilibrium fugacity of CO₂ in surface ocean water of the tropical limbs of the North Pacific (a) and the South Pacific (b) subtropical gyres. The regional mean measured seawater fugacities are plotted as solid circles, and the fugacities of atmospheric CO₂ at the water-saturated air-sea interface are connected by dotted lines. Surface ocean water fugacities predicted from temperature and salinity without air-sea exchange are shown as dashed lines, and with air-sea exchange are shown as solid lines (see text).

Table 1 Mean properties of surface ocean water and the atmosphere during the NORPAX equatorial experiment

Leg no.	Date	Seawater f_{CO_2} (μatm)	t ($^{\circ}\text{C}$)	S (‰)	Normalized A_T^* ($\mu\text{equiv. kg}^{-1}$)	Atmosphere $f_{\text{CO}_2}^\dagger$ (μatm)
North Pacific subtropical gyre (15–20° N, 158° W)						
5	14 July 1979	—	25.99	34.477	—	327.6
7	10–11 September 1979	341.3	27.11	34.682	2,319.8	322.0
9	23–24 November 1979	329.8	26.31	34.685	2,317.9	323.9
11	2–3 February 1980	—	25.51	34.737	—	327.0
13	9–10 April 1980	319.9	25.13	34.722	2,322.0	329.5
15	13–14 June 1980	333.2	25.98	34.672	2,317.7	328.8
South Pacific subtropical gyre (14–17° S, 150° W)						
5	18 June 1979	—	27.58	35.798	—	322.9
7	19 August 1979	318.6	27.03	36.080	2,321.6	324.4
9	1 November 1979	317.6	26.97	36.099	2,319.7	322.2
11	8 January 1980	—	28.54	35.737	—	322.4
13	18 March 1980	333.3	29.09	35.696	2,319.9	321.9
15	18 May 1980	328.3	28.94	35.481	2,317.2	321.9

* Mean values of A_T calculated from measured ΣCO_2 and f_{CO_2} (see text) and normalized to $S = 35\%$.

† Fugacities at the water-saturated air-sea interface.

with the calibration of compressed air standards by nondispersive IR analysis and manometric techniques², is $\sim 0.05\%$. An additional estimated uncertainty of 0.5% is associated with possible systematic effects in the seawater equilibration process.

Measurements of atmospheric CO_2 were also made by shorebased nondispersive IR analysis² using flask samples collected in triplicate on all the NORPAX expedition legs. The standard deviation of replicate flask measurements is $\sim 0.06\%$, with an additional systematic uncertainty of $\sim 0.04\%$.

Seawater samples for shorebased determination of total inorganic carbon (ΣCO_2) were collected at hydrographic stations on northbound odd-numbered expedition legs using Niskin bottles at a depth of 10 m. The samples were collected in duplicate and were preserved with HgCl_2 . The ΣCO_2 measurements were made by acidifying a weighed sample and trapping the evolved CO_2 with liquid nitrogen in a vacuum system. The CO_2 was separated from H_2O with a dry ice trap and was then determined manometrically with the same constant volume manometer used to determine the mole fraction of CO_2 in air². The standard deviation of replicate samples is $0.7 \mu\text{M kg}^{-1}$, and the accuracy, as judged from artificial samples, is $\sim 1.0 \mu\text{M kg}^{-1}$.

The measured concentrations of CO_2 in the equilibrated gas and in the atmosphere were converted to fugacities for the purpose of evaluating real-gas equilibrium relationships, using the virial equation of state³ and assuming a water-saturated interface at the measured barometric pressure. The solubility of CO_2 in seawater as a function of fugacity was taken from the equations in ref. 3. Calculations of carbonate system equilibria were carried out using the dissociation constants in seawater of carbonic acid, boric acid and water, as measured on the hydrogen ion concentration scale by Hansson⁴ and fitted to the equations of Millero⁵. These values were chosen in preference to the measurements on the NBS buffer scale^{6–8}, principally because the Hansson values for boric acid are in better agreement with the work of Millero⁹ and with the recent measurements of A. Dickson (personal communication) than are the Lyman values⁷. Note, however, that the results presented below are not strongly dependent on the choice of dissociation constants².

The temperature dependence of the fugacity of CO_2 (f_{CO_2}) in a gas phase in thermodynamic equilibrium with seawater at constant total titration alkalinity (A_T) and constant ΣCO_2 was determined empirically from calculated equilibria. Values of f_{CO_2} were calculated over the ranges of $0\text{--}36^{\circ}\text{C}$ in temperature (t), $30\text{--}38\%$ in salinity (S), $64\text{--}68 \mu\text{equiv. kg}^{-1} \%$ in specific alkalinity (A_T/S), and $1.05\text{--}1.20 \mu\text{equiv. } \mu\text{M}^{-1}$ in the $A_T/\Sigma\text{CO}_2$ ratio. The resulting values of f_{CO_2} bracket the atmospheric range at all temperatures and salinities and include extrema from 80 to 2,000 μatm (atm). For each combination of ΣCO_2 , A_T and S , $\ln(f_{\text{CO}_2})$ was fitted by least squares to a power series in t . These expressions were then differentiated

to obtain values of $\partial \ln(f_{\text{CO}_2})/\partial t$, which were again fitted to the equation

$$\frac{\partial \ln(f_{\text{CO}_2})}{\partial t} = 0.03107 - 2.785 \times 10^{-4} t - 1.839 \times 10^{-3} \ln(f_{\text{CO}_2}) \quad (1)$$

which represents the temperature dependence of the calculated fugacities with a standard deviation of $0.05\% ^{\circ}\text{C}^{-1}$. The precision of this fit greatly exceeds those of previous empirical representations¹⁰.

The salinity dependence of f_{CO_2} at constant specific alkalinity and constant specific total inorganic carbon ($\Sigma\text{CO}_2/S$) was determined in an analogous fashion to the temperature dependence, using values of f_{CO_2} calculated over the same ranges of t , S , A_T , and $A_T/\Sigma\text{CO}_2$. For each combination of t , A_T/S and $\Sigma\text{CO}_2/S$, $\ln(f_{\text{CO}_2})$ was fitted to a power series in S , differentiated to obtain values of $\partial \ln(f_{\text{CO}_2})/\partial S$, and fitted to the equation

$$\frac{\partial \ln(f_{\text{CO}_2})}{\partial S} = 0.08620 - 1.272 \times 10^{-3} S \quad (2)$$

which represents the salinity dependence of the calculated fugacities with a standard deviation of 0.06% per $\%$ salinity.

An important characteristic of equations (1) and (2) is their independence from A_T and ΣCO_2 , which makes it possible to evaluate the effects of temperature and salinity variations on f_{CO_2} in nature and in experimental applications without the need to determine other parameters of the dissolved carbonate system. Both equations are easily integrated in modelling applications, without significant loss in accuracy compared with equilibrium calculations for waters with well-characterized carbonate systems.

The results of the NORPAX f_{CO_2} measurements in the North and South Pacific subtropical gyres are summarized in Table 1. The mean values listed are confined to the tropical limbs of the gyres north of 15°N and south of 14°S , where the continuous chromatographic measurements do not show the increase in f_{CO_2} due to upwelling in the equatorial region¹¹. To facilitate comparisons between the chromatographic measurements and the hydrographic observations spaced at 1° latitude intervals¹², the tabulated values include only the chromatographic measurements at the individual station locations. In addition to the data for expedition Legs 7, 9, 13 and 15, on which the chromatographic measurements were made, Table 1 includes hydrographic and atmospheric CO_2 flask data for adjoining Legs 5 and 11.

The seasonal variations of surface water f_{CO_2} in both gyres are plotted in Fig. 1. The peak-to-peak amplitude of the variations is about $20 \mu\text{atm}$, with both hemispheres showing summer maxima. The predicted variations in f_{CO_2} , calculated from the observed changes in temperature and salinity by integration

of equations (1) and (2) with the fugacity boundary condition adjusted to minimize the r.m.s. deviation of the measured fugacities, are plotted for each hemisphere as dashed lines in Fig. 1. The agreement between the measurements and these calculations, which do not allow for air-sea exchange or for seasonal variations in the admixture of waters with other carbonate chemistry, is nonetheless striking, with r.m.s. deviations of 3.0 and 2.3 μatm in the northern and southern gyres respectively. Thus, the observed variations in both hemispheres are due mainly to seasonal variations in surface water temperature and salinity, rather than to removal or addition of CO_2 by physical or biological processes.

We have considered the effect on f_{CO_2} of biological activity, as reflected in the surface water nutrient concentrations¹². Assuming carbonate chemistry equilibrium, the Redfield, Ketchum and Richards mean planktonic composition¹³, and the complete assimilation of available nitrate, the maximum predicted f_{CO_2} variation is 0.3 μatm in the northern gyre and 0.6 μatm in the southern gyre. However, inasmuch as the measured surface water nitrate values in these regions remain at or below the 0.1 $\mu\text{M kg}^{-1}$ detection limit, we have neglected this small effect in our model calculations.

The solid lines in Fig. 1 show the effect of including air-sea exchange in the calculations. This was done by a simple numerical integration, in which the CO_2 flux was assumed to be proportional to the fugacity difference between the surface water and the measured atmosphere, the mean exchange rate was taken as 22 $\text{M m}^{-2} \text{yr}^{-1}$ (refs 14, 15), the mean depth of the mixed layer determined from the hydrographic data was 70 m, the mean values of t , S and atmospheric f_{CO_2} were interpolated linearly between legs, and the mean buffer factor $\partial \ln(f_{\text{CO}_2}) / \partial \ln(\Sigma \text{CO}_2)$ calculated from the chemical constants discussed above was 8.35 in the northern gyre and 8.50 in the southern gyre. As in the case of the conservative calculation, the fugacity boundary condition in each hemisphere was adjusted to minimize the r.m.s. deviation of the measured seawater fugacities. The main effect of adding air-sea exchange to the calculations is a slight advance in the phase of the predicted variations, which further reduces the r.m.s. deviations to 2.6 and 1.1 μatm in the northern and southern gyres respectively.

This excellent agreement between the measured values of f_{CO_2} and the predicted variations for a model which is conservative in CO_2 except for air-sea exchange, requires that the salinity-normalized alkalinity in both gyres be constant to within about 2 $\mu\text{equiv. kg}^{-1}$. To test this hypothesis, we have calculated A_T from our ΣCO_2 and f_{CO_2} measurements using the chemical constants discussed above. The mean values of A_T normalized to $S = 35\%$ are listed in Table 1. The averages and standard deviations of the normalized values of A_T for the four expedition legs are $2,319.3 \pm 2.0 \mu\text{equiv. kg}^{-1}$ in the northern gyre and $2,319.6 \pm 1.8 \mu\text{equiv. kg}^{-1}$ in the southern gyre. The results thus confirm the remarkable seasonal constancy of specific alkalinity in the tropical limbs of the Pacific subtropical gyres, and demonstrate that seasonal variations in their CO_2 fugacities may be calculated directly from changes in temperature and salinity, with a small correction for air-sea exchange.

Note that the seasonally averaged surface water CO_2 fugacities in the tropical limbs of both gyres are significantly greater than the seasonally averaged atmospheric fugacities (Fig. 1), and there is therefore a net flux of CO_2 from the sea to the atmosphere of the order of $0.1 \text{ M m}^{-2} \text{yr}^{-1}$. While part of this flux may be due to upwelling across the thermocline, we expect the major source to be thermal pumping of atmospheric CO_2 which dissolves in the cooler high-latitude limbs of the gyres and is subsequently liberated as these surface waters are advected into the tropics and warmed. However, seasonal measurements of CO_2 in higher latitudes are needed to determine the relative importance of these mechanisms.

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A new type of decollement thrusting

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One of the fundamental rules of decollement tectonics is that decollement horizons form in mechanically weak layers^{1,2}. Here we document two examples of decollement-style thrust faults that detach within thick platform dolostones in preference to apparently weaker layers of shale, siltstone and limestone underlying the dolostones. The thrusts are the Keystone-Muddy Mountain-Glendale thrust system (the KMG thrust system), and the underlying Contact-Red Spring-North Buffington-Mormon thrust system (the CRM thrust system), both of southern Nevada. They form part of the Sevier orogenic belt, and extend for roughly 250 km along strike, together showing at least 65 km tectonic overlap (Fig. 1). Although the thrust systems are closely related spatially, the higher KMG system is younger than the underlying CRM thrust system, and the two developed essentially independently³⁻⁷. Three points are important: first, that both the KMG and the CRM thrust systems are decollement style thrusts; second, that the decollement horizon is primarily restricted to a narrow stratigraphical interval within a bedded sequence of essentially homogeneous dolostones of the Middle Cambrian Bonanza King Formation; the third, that the thrust faults formed at a very shallow crustal level (<5 km).

Detailed field studies in the past 15 yr have yielded a wealth of structural and stratigraphical data for the thrust systems in question⁵⁻¹². To demonstrate a decollement geometry for any foreland thrust, it is sufficient to show that the thrust fault carries the same structural or stratigraphical unit in the hanging wall for a large distance across strike (in the transport direction). If this criterion is met, the thrust fault must have decollement geometry, regardless of later deformations. The two thrust systems are discussed separately—first the CRM thrust system, then the KMG thrust system.

Both the KMG thrust system and the CRM thrust system carry the Banded Mountain Member of the Bonanza King

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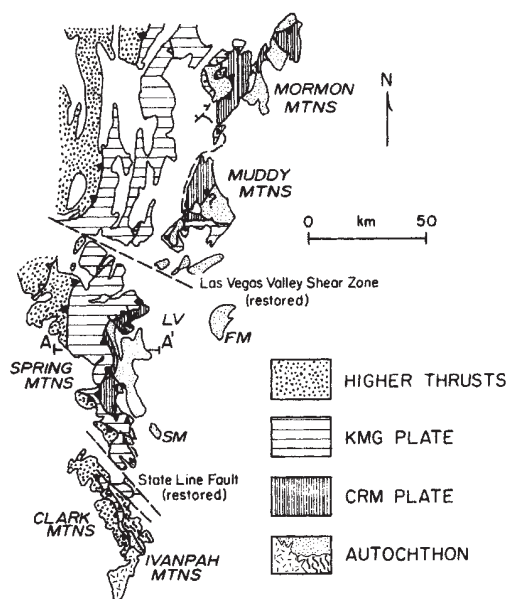


Fig. 1 Simplified tectonic map of the Keystone-Muddy Mountain-Glendale and Contact-Red Spring-North Buffington-Mormon thrust systems. Right-lateral movement on the Tertiary Las Vegas Valley shear zone and Pahrump-State Line fault zone has been removed. Thrust transport direction is roughly east-northeast⁹. LV, Las Vegas; FM, Frenchman Mountain; SM, Sheep Mountain.

Formation in the hanging wall for great distances along and across strike. With a few exceptions, the thrust faults virtually everywhere occur within 75 m of a distinctive marker horizon at the base of the Banded Mountain Member^{10,11,13}. The Middle and Upper Cambrian Bonanza King Formation (for original definition, see ref. 14) is the basal unit of the thick Palaeozoic miogeoclinal platform carbonate sequence of the southern Great Basin. Two members are commonly identified: the upper Banded Mountain Member and the lower Papoose Lake Member^{9,13,15}. The Banded Mountain Member generally consists of well-bedded dolostone with rare limestone in the region of Fig. 1; the Papoose Lake Member generally consists of mottled dolostone and limestone¹³. The Banded Mountain Member averages ~500 m in thickness where a complete section is exposed; the Papoose Lake Member varies between 175 and 450 m thick, where exposed (Fig. 2).

The Banded Mountain Member forms the hanging wall of the Mormon thrust fault for a distance of at least 40 km in the transport direction⁷. The Mormon thrust fault is probably correlative with the North Buffington thrust in the central Muddy Mountains, and the Red Spring and Contact thrusts in the central Spring Mountains^{6,7}. All of these correlative thrusts carry the Banded Mountain Member of the Bonanza King Formation in the hanging wall^{6,12}. The CRM thrust system carries the same unit in the hanging wall for at least 40 km across strike and at least 150 km along strike; it therefore has a decollement geometry.

Thrust faults in the KMG thrust system carry the Banded Mountain Member of the Bonanza King Formation in the hanging wall for 15 km across strike in the northern Clark Mountains^{5,8}, for 8–10 km across strike in the central Spring Mountains^{10,16}, and for at least 25 km across strike in the Muddy Mountains⁶. The KMG thrust system carries the Banded Mountain Member in the hanging wall for nearly 200 km along strike, from the southern Mormon Mountains in the north⁷, through the Muddy Mountains⁶ and Spring Mountains^{5,9} to the Clark and Ivanpah Mountains of southeastern California⁸. Therefore, the KMG thrust system also has decollement geometry, by the criterion above.

The phrase 'in the hanging wall' is used to denote that part of the upper plate immediately adjacent to the thrust fault itself: 'hanging wall' means the bottom surface of the upper plate of the thrust, and not volume of the upper plate.

Because the Banded Mountain Member forms the hanging wall for virtually all of the exposures of both thrust systems, it is clear that this unit contains the decollement horizon for the exposed portions of the thrusts. To demonstrate that a decollement horizon formed within the Bonanza King Formation in preference to the underlying Middle and Lower Cambrian limestones, shales and siltstones, it must be shown that the Papoose Lake Member was present beneath the Banded Mountain Member in the allochthon before formation of the thrust faults.

Complete sections of Cambrian strata are exposed in the autochthon in the Mormon Mountains, at Frenchman Mountain, at Sheep Mountain and in the Ivanpah Mountains (see Fig. 1). All three of these sections contain a thick Papoose Lake Member^{7,9,17,18}. The Papoose Lake Member is also present in the higher allochthons west of the KMG thrust system^{8,9,19,20}. The Papoose Lake Member and underlying strata are regionally extensive, present everywhere the entire Cambrian section is exposed both east and west of the two thrust systems discussed here. It follows that these beds were originally present beneath the Banded Mountain Member of both the KMG and CRM allochthons, and were left behind beneath the decollement during thrust faulting. This is supported by the occurrence of slivers of the Papoose Lake Member preserved locally above the thrusts^{12,18}.

It is possible that the decollement in the Banded Mountain Member is not a basal decollement, but rather the roof fault for a duplex structure at depth. There is no structural evidence for such a duplex: if one exists it is nowhere exposed, and an internally consistent set of balanced cross-sections can be drawn without postulating such a structure. In addition, the CRM thrust system, overlying KMG thrust system, and still higher Green Monster-Lee Canyon thrust system all carry the basal beds of the Banded Mountain Member in the hanging wall in virtually every exposure; the Papoose Lake Member is present only as slivers, and lower units are not present at all. The simplest explanation is to postulate a master decollement near the base of the Banded Mountain Member.

We infer that the CRM and the KMG thrust systems formed at shallow levels on the basis of the general absence of ductile deformation within the thrust sheets and the presence of fore-thrust debris and stream-channel conglomerates in the footwall of both thrust systems. The absence of pervasive ductile deformation

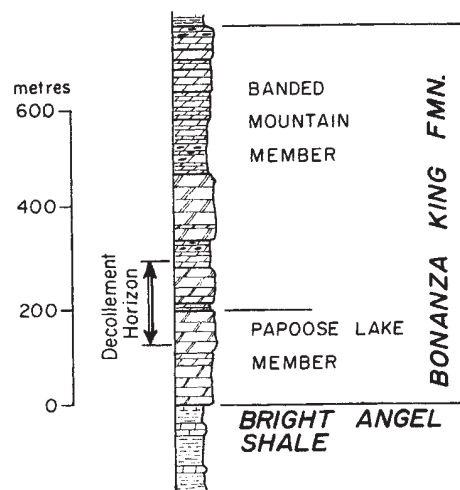


Fig. 2 Generalized stratigraphical column of the Bonanza-King Formation in the area of Fig. 1. Decollement horizon of the Keystone-Muddy Mountain-Glendale and Contact-Red Spring-North Buffington-Mormon thrust systems lies at base of the Banded Mountain Member.

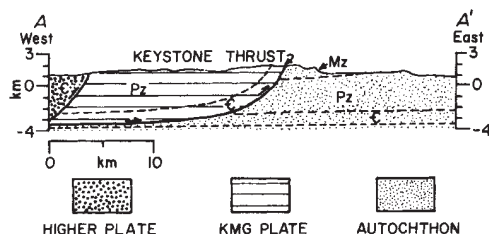


Fig. 3 Cross-section of the Keystone thrust system in the central Spring Mountains (A–A' in Fig. 1), showing decollement nature of the fault and simple ramp geometry. No vertical exaggeration.

mation merely implies that the thrusts did not form in metamorphic conditions. The presence of forethrust debris beneath the thrust faults implies that both thrust systems were 'erosional thrusts'; thrust systems whose fault plane lay at or near the surface of the Earth during thrusting, and whose upper surface was a topographic surface during thrust advance^{21–23,30}. A typical cross-section through the central Spring Mountains (Fig. 3) shows that the Keystone thrust fault forms a single ramp from the decollement horizon in the Banded Mountain Member of the lower plate to the ancient erosional surface. The autochthonous section is roughly 4.5 km thick at the Keystone ramp; therefore the thrust fault at the base of the ramp must have been no more than 5–5.5 km below the surface at the time of formation, allowing for post-Keystone erosion. A similar argument holds for the CRM thrust system.

One important aspect of the Banded Mountain Member decollement surface is its very large area. Palinspastic restoration of the displacements on the two thrust systems provides an estimate of the initial size of the decollement surface. The length along strike is at least 200 km, from exposures of the KMG thrust system. Adding values of tectonic overlap for the KMG and CRM thrust systems will give a minimum value for the across strike extent of the decollement surface. Minimum overlap on the CRM system is 40 km (ref. 7); minimum overlap on the KMG system is 25 km (ref. 6). The decollement formed in the Banded Mountain Member must then be at least 65 km wide; the total area of the decollement must be at least 13,000 km².

The principal mechanism of deformation near the thrust faults appears to be cataclasis²⁴. Moderate to intense brecciation is common in the upper plate up to several tens of metres away from the fault^{7,8,10–12,18,25}. Evidence for extensive ductile deformation within the thrust sheets is present only in the Clark Mountains at the southernmost end of the Keystone thrust, where late syn-tectonic plutonism provides a source for heat and fluids^{8,18}. Evidence for ductile deformation near the thrust fault disappears a short distance away from the plutons.

Of paramount importance in the problem of formation of this decollement surface is that there are no lithological reasons for postulating any special mechanical properties for the rocks of the Banded Mountain Member in the decollement zone: there are no shale layers, no gypsiferous layers, nothing but monotonous fine-grained laminated dolostone locally including secondary, recrystallized coarse-grained dolomite (Fig. 2). This has been established by detailed stratigraphical and petrological studies in both allochthonous and autochthonous sections^{5,16}. The formation of the decollement horizon appears to be controlled by factors other than lithological contrasts within the decollement zone.

The key mechanical problem is not the transport of these thrust systems, although that problem is complex, and despite recent purely theoretical models^{23,26} is still poorly understood. The main mechanical problem is: why did the decollement form within the dolostones, ignoring shale only a few hundred metres below? Our present view is that when subjected to horizontal compression the highly competent dolostones acted as a stress guide, concentrating horizontal stress within the dolostones unit with respect to the weaker materials below²⁷. Preliminary

modelling studies by one of us (J.H.W.) indicate that this variation in horizontal stress may have a key role in the localization of the decollement horizon.

The observation that the thrusts formed at shallow crustal levels is very important in light of the low strength of shale, siltstone and limestone in brittle conditions²⁸. Our finding that the decollement formed within the strong dolostones in preference to the apparently weaker underlying layers demonstrates that a low-strength layer at the decollement horizon is neither a necessary nor a sufficient condition for the formation of a decollement thrust in the orogenic foreland. Existing theories of foreland thrusting appear inadequate to explain the formation of these decollement zones^{2,29}. We suggest that the Keystone–Muddy Mountain–Glendale thrust system and the Contact–Red Spring–North Buffington Mormon thrust system represent type examples of a class of 'strong decollement' foreland thrusts.

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The La–Ce geochronometer: a new dating method

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The isotope ¹³⁸La (0.089% of natural lanthanum) decays by β^- and electron capture to ¹³⁸Ce and ¹³⁸Ba with a long half-life. We present here the first age determination using La–Ce dating. An internal La–Ce isochron of a gabbro from the upper zone of the Bushveld, South Africa, yields an age of $2,390 \pm 480$ (2 σ) Myr based on the partial decay constant $2.58 \times 10^{-12} \text{ yr}^{-1}$. This age agrees with the Sm–Nd isochron age ($2,050 \pm 90$ (2 σ) Myr) which was obtained from an aliquot of the same sample solution: within the uncertainties of ¹³⁸La half life and isotopic measurement.

Radioactivity of ¹³⁸La has been predicted, because of Mat- tauch's rule concerning the stability of isobaric atomic nuclei

Table 1 Summary of partial and total half life data and radioactivity determination of ^{138}La

Partial half life (10^{11} yr)	EC decay		Total half life (10^{11} yr)	Method	Refs
	β decay	β decay			
12	0.7	0.06	2.0	γ counting with NaI	2
2.4 ± 0.2	2.1 ± 0.1	0.88 ± 0.08	0.7	β counting with Al absorbent	3
3.5 ± 0.3	1.64 ± 0.06	0.47 ± 0.04	1.0 ± 0.1	γ counting with NaI	4
4.1 ± 0.7			1.12 ± 0.10	γ counting with NaI	5
2.83 ± 0.04	1.66 ± 0.02	0.59 ± 0.01	1.04 ± 0.014	β counting with proportional tube	5
4.7 ± 1.5	2.34 ± 0.37	0.50 ± 0.18	1.56 ± 0.3	γ counting with NaI	6
3.95 ± 0.63	1.87 ± 0.21	0.47 ± 0.09	1.27 ± 0.18	γ counting with Ge(Li)	7
3.68 ± 0.14	1.99 ± 0.03	0.54 ± 0.02	1.29 ± 0.05	γ counting with Ge(Li)	8
		0.51 ± 0.04	1.28 ± 0.12	γ counting with Ge(Li)	9
3.02 ± 0.04	1.56 ± 0.05	0.51 ± 0.01	1.03 ± 0.02	γ counting with Ge(Li)	10
$2.95 \pm 0.15^*$	1.74 ± 0.13		1.05 ± 0.05	γ counting with Ge(Li) and pure Ge	11
$2.69 \pm 0.24^\dagger$	1.51 ± 0.10		0.94 ± 0.06		

* Weighted mean of the data from refs 4–11.

† Weighted mean of the data from refs 4–10 which were multiplied by 1/1.17 (see text) and raw data of ref. 11.

(^{138}Ba – ^{138}La – ^{138}Ce) with contiguous atomic numbers and the half-life has been measured^{11–11,25}. ^{138}La decays by β^- emission to an excited level of ^{138}Ce which emits 789 keV γ ray and by electron capture to an excited level of ^{138}Ba which emits a 1,436 keV γ ray. The published values for the half lives of these two decay processes and weighted average values are summarized in Table 1. These partial half lives as well as the total half life scatter rather widely, while the branching ratios of the two decays are in reasonable agreement. Reported values of half lives with no information on volatile impurities were uniformly reduced here by a factor of 0.855 (=1/1.17) assuming that the oxide measured contained 17% volatiles due to absorption of H_2O and CO_2 . The weighted average values are also listed in Table 1.

An upper zone gabbro of the Bushveld complex was sampled at Magnet Height, is composed of about 50% pyroxenes, 40% plagioclase and minor amounts of biotite, hornblende and magnetite. The gabbro was crushed and pyroxenes and plagioclase were separated from both 60–80 and 80–140 mesh fractions by isodynamic separator.

About 0.5–1 g for each sample (two pyroxenes, two plagioclases and a bulk rock) was decomposed by HF and HClO_4 . The samples were then dissolved in HCl and separated into two aliquots, and composite REE spike was added to one of them. Ce and Nd were chemically separated for the measurement of isotopic abundance ratios involved three ion-exchange columns, and, Nd was finally eluted by 0.25 M α -hydroxyisobutyric acid (α -HIBA), and Ce by 0.3 M α -HIBA, respectively. Overall blanks were 0.4 ng for Nd and 3 ng for Ce. The moderately high blanks were due to the large amounts of reagents used for large samples. However, even for the most Ce-poor pyroxene, the sample/blank ratio was more than 800 for both elements, and hence no correction for blanks on isotopic ratio was made. Blank values for abundance determination by isotope dilution were 10 times smaller than those for isotopic composition. Tiny CeO^+ peaks with natural isotopic ratios were detected from the outgassed filament system, and

the beam intensity of mass 158 ($^{142}\text{Ce}^{16}\text{O}$) was $\sim 5 \times 10^{-16}$ A in the same operating condition as normal running with a sample. This background intensity was less than 1/10,000 of the sample beam intensity, and the effect was included in the blank value mentioned above.

Cerium isotopes were measured as CeO^+ on Re triple filaments. The peaks of masses 152 ($^{136}\text{Ce}^{16}\text{O}$), 154 ($^{138}\text{Ce}^{16}\text{O}$) and 158 ($^{142}\text{Ce}^{16}\text{O}$) were measured. The biggest peak of mass 156 ($^{140}\text{Ce}^{16}\text{O}$) was ignored in order to get good measurements for the tiny peaks of masses 152 and 154. Masses 153, 155, 157 and 162 were usually monitored. Although the small 155 ($^{139}\text{La}^{16}\text{O}$) and 157 ($^{141}\text{Pr}^{16}\text{O}$) peaks were observed in most cases, the 154/155 and 158/157 intensity ratios were greater than 50 and 1,000, respectively. Very small BaO^+ and BaF^+ peaks were detected only at an early low temperature stage and soon disappeared with growth of the CeO^+ beam. NdO^+ was observed in a few cases and corrected where necessary. The beam intensity of mass 158 ($^{142}\text{Ce}^{16}\text{O}$) was 0.5 to 1.0×10^{-11} A. Generally 100 scans of the peaks were accumulated for each run. Each run took 3–4 h and 2–4 runs were measured for every sample. $^{136}\text{Ce}/^{142}\text{Ce} = 0.01720$ was used for normalization. The observed fractionation factor between ^{136}Ce and ^{142}Ce was used to estimate the $^{140}\text{Ce}/^{142}\text{Ce}$ ratio, followed by an evaluation of $^{140}\text{Ce}^{18}\text{O}^+$ effect on $^{142}\text{Ce}^{16}\text{O}^+$. $^{140}\text{Ce}/^{142}\text{Ce}$ was taken to be 7.992 at $^{136}\text{Ce}/^{142}\text{Ce} = 0.01720$, that is, with no mutual fractionation. However, the mass fractionation correction for $^{140}\text{Ce}^{18}\text{O}$ was very small, and almost constant. The Johnson Matthey Ce reagent, JMC 304, was measured with a rather wide range of ion current ($0.3 \sim 2 \times 10^{-11}$ A) and isotope fractionation [$(^{136}\text{Ce}/^{142}\text{Ce})$ fractionation = $1.0001 \sim 0.996$]. No systematic variation was found. The mean $^{138}\text{Ce}/^{142}\text{Ce}$ value for 15 runs on the reagent was 0.0228559 ± 0.0000011 (2 σ).

Neodymium was measured as NdO^+ by a Re single filament. Small PrO^+ and SmO^+ peaks were sometimes observed and $^{147}\text{Sm}^{16}\text{O}$ was monitored for Sm correction if needed. All the measured isotopic ratios were normalized against $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$. La Jolla Nd Standard reagent (provided

Table 2 La–Ce and Sm–Nd isotopic data for Bushveld upper zone gabbro

Sample	La (p.p.m.)	Ce (p.p.m.)	$^{138}\text{La}/^{142}\text{Ce}^*$	$^{138}\text{Ce}/^{142}\text{Ce}^\dagger$	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}^*$	$^{143}\text{Nd}/^{144}\text{Nd}^\dagger$
60–80 mesh fraction								
Pyroxene	1.010	3.91	0.00209	0.022848 ± 4	1.255	3.81	0.1993	0.51230 ± 5
Plagioclase	2.39	3.74	0.00518	0.022868 ± 9	0.1391	1.123	0.0749	0.51066 ± 7
80–140 mesh fraction								
Pyroxene	0.640	3.01	0.00172	0.022843 ± 7	0.989	2.97	0.2014	0.51235 ± 5
Plagioclase	2.35	3.71	0.00514	0.022864 ± 8	0.1350	1.082	0.0755	0.51062 ± 7
Bulk	1.743	4.23	0.00334	0.022856 ± 5	0.689	2.53	0.1647	0.51189 ± 4

* $^{138}\text{La}/^{142}\text{Ce} = (\text{La}/\text{Ce})_{\text{weight}} \times 0.00811$. The factor was based on ^{138}La abundance (0.00089), ^{140}Ce abundance (0.1107) and atomic weights 138.91 and 140.12 for La and Ce, respectively^{25,26}. The factor 0.6049 was used for $^{147}\text{Sm}/^{144}\text{Nd}$ (ref. 27).

† Errors are $2\sigma_m$.

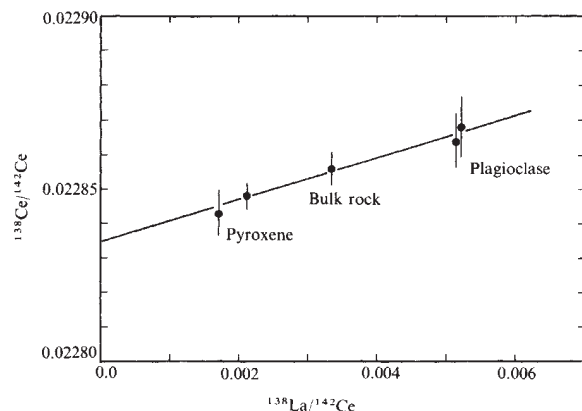


Fig. 1 La-Ce mineral isochron plot for an upper zone gabbro of the Bushveld complex, South Africa. The isochron yields an age of $2,390 \pm 480$ (2 σ) Myr with an initial $^{138}\text{Ce}/^{142}\text{Ce}$ ratio of 0.022835 ± 0.000004 (2 σ) ($\lambda_{\beta}^{138}\text{La} = 2.58 \times 10^{-12} \text{ yr}^{-1}$).

by G. W. Lugmair) was used as a reference standard. Our $^{143}\text{Nd}/^{144}\text{Nd}$ ratio was 0.51183 ± 0.00003 (2 σ). All seven Nd isotopes for samples were measured to check the reliability of $^{143}\text{Nd}/^{144}\text{Nd}$ measurement. Most $^{142}\text{Nd}/^{144}\text{Nd}$ and $^{145}\text{Nd}/^{144}\text{Nd}$ ratios agreed with those of the standard reagent within $\pm 1\epsilon$, and $^{148}\text{Nd}/^{144}\text{Nd}$ and $^{150}\text{Nd}/^{144}\text{Nd}$ ratios agreed with the standard within $\pm 2\epsilon$. A Micromass 30-54R was used for Ce and Nd measurement.

Results are shown on Table 2 and in Figs 1 and 2. The isochron lines were fitted using the York method¹². Uncertainties (2 σ) for $^{138}\text{La}/^{142}\text{Ce}$ and $^{147}\text{Sm}/^{144}\text{Nd}$ were estimated at 1% and 0.5% respectively. Assuming a partial half life of $2.95 \times 10^{11} \text{ yr}$ ($\lambda_{\beta} = 2.35 \times 10^{-12} \text{ yr}^{-1}$) for ^{138}La (Table 1), the La-Ce isochron yields an age of $2,630 \pm 530$ Myr (2 σ). Similarly, using the modified partial half life of $2.69 \times 10^{11} \text{ yr}$ ($\lambda_{\beta} = 2.58 \times 10^{-12} \text{ yr}^{-1}$) in Table 1 yields an age of $2,390 \pm 480$ Myr. The former age, $2,630 \pm 530$ Myr, seems slightly older than the Sm-Nd age ($2,050 \pm 90$ Myr (2 σ)) with an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50964 ± 0.00010 (2 σ); the latter age, $2,390 \pm 480$ Myr obtained with the modified decay constant is in better agreement with the Sm-Nd age. This suggests that the absorbed H_2O and CO_2 correction in the decay constant measurement as discussed above, was pertinent and that the modified decay constant in Table 1 is more appropriate.

The Bushveld mafic phase has been studied by several investigators. Ages cited here were recalculated on the basis of the new decay constants¹³. Separated biotite from a gabbro of Rustenburg Plats gave a Rb-Sr age of $2,007 \pm 49$ Myr (2 σ) (ref 14). Hamilton¹⁵ determined the Rb-Sr whole rock ages systematically from lower zone to upper zone. These ages are in reasonable agreement through the layer of the mafic rock phase, ranging from $1,901 \pm 16$ (2 σ) to $2,126 \pm 61$ (2 σ) Myr. A biotite-plagioclase-whole rock internal isochron age, $2,148 \pm 14$ Myr, is in agreement with the whole-rock isochron ages. Hamilton averaged three selected isochron ages, that is $2,091 \pm 94$ Myr from the Upper Zone, $2,095 \pm 14$ Myr from the Merensky Reef and $2,035 \pm 18$ Myr from the Transition Zone, and presented an age of $2,050 \pm 23$ Myr as the most reliable for the Bushveld mafic phase. La-Ce and Sm-Nd ages agree with published Rb-Sr ages, although the experimental uncertainty of La-Ce age is considerable.

The $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of chondritic uniform reservoir (CHUR) at 2,050 Myr ago was calculated to be 0.51002 by using $^{143}\text{Nd}/^{144}\text{Nd}$ ratio (0.50682) of the achondrite Angra dos Reis¹⁶, Sm/Nd ratio (0.321 in weight)¹⁷ of Leedey chondrite and the decay constant ($6.54 \times 10^{-12} \text{ yr}^{-1}$) of ^{147}Sm . The initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio, 0.50964 ± 0.00010 (2 σ), of the Bushveld upper zone gabbro is slightly lower than the value of the CHUR at 2,050 Myr ago. This fact suggests that the gabbro was derived from a slightly light REE (also probably large ionic lithophiles (LIL)) enriched source compared with CHUR. This is consistent

with the Sr data. Two initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for the upper zone gabbro were obtained as 0.70769 ± 0.00006 (2 σ) and 0.70735 ± 0.00008 (2 σ) (ref.15). These ratios suggest that the gabbro was derived from LIL enriched source as deduced from the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio. Coherent variation of La-Ce and Sm-Nd as a rare earth group suggests that the initial $^{138}\text{Ce}/^{142}\text{Ce}$ ratio ($R_{\text{gabbro}}^{\text{Ce}}$ (2,390 Myr)), 0.022835 ± 0.000004 (2 σ), must be higher than the $^{138}\text{Ce}/^{142}\text{Ce}$ of CHUR ($R_{\text{CHUR}}^{\text{Ce}}$ (2,390 Myr)) at that time.

Our results confirm that the La-Ce isotopic system is a promising new geochronometer. Moreover, the La-Ce system, coupled with the Sm-Nd system, will become a unique tool for isotope geochemistry, because both pairs belong to a rare earth group, and this is expected to throw a new light on those studies. In the following section, we will show the variation of $^{138}\text{Ce}/^{142}\text{Ce}$ for some terrestrial materials and meteorites.

As discussed above, the gabbro is considered to be derived from a slightly more radiogenic source on ^{138}Ce than CHUR; $R_{\text{gabbro}}^{\text{Ce}}$ (2,390 Myr) $>$ $R_{\text{CHUR}}^{\text{Ce}}$ (2,390 Myr). However, it is reasonable to assume that Ce in the source of the gabbro was less radiogenic than the continental crust. Then, $R_{\text{CHUR}}^{\text{Ce}}$ (4,550 Myr) should be lower than the value obtained by tracing back the $R_{\text{gabbro}}^{\text{Ce}}$ (2,390 Myr) to $T = 4,550$ Myr by retrograde decay correction with assuming chondritic La/Ce ratio (0.387 by weight), while it should be higher than the value obtained by tracing back the $R_{\text{gabbro}}^{\text{Ce}}$ (2,390 Myr) to $T = 4,550$ Myr by retrograde decay correction assuming the crustal La/Ce ratio (the value of North American Shale, 0.457 was used)¹⁸. Then, $R_{\text{CHUR}}^{\text{Ce}}$ (4,550 Myr) is calculated to be higher than 0.022814 and lower than 0.022817 by using $\lambda_{\beta}^{138}\text{La} = 2.58 \times 10^{-12} \text{ yr}^{-1}$. The La/Ce of the source region of the gabbro is expected to be closer to chondritic than crustal in chemical composition and in the La/Ce ratio. Then we choose rather arbitrarily 0.022816 as a $R_{\text{CHUR}}^{\text{Ce}}$ (4,550 Myr) between 0.022814 and 0.022817.

The ϵ notation is used as it is generally on Sr and Nd. $^{138}\text{Ce}/^{142}\text{Ce}$ ratios are given as fractionated deviations in parts per 10^4 (ϵ_{Ce}) from the value in the CHUR and are given by $\epsilon_{\text{Ce}}(T) = [R_{\text{s}}(T)/R_{\text{CHUR}}(T) - 1] \times 10^4$ where $R_{\text{s}}^{\text{Ce}}(T)$ is the $^{138}\text{Ce}/^{142}\text{Ce}$ of a sample (s), T is the age under consideration and $\lambda_{\beta}^{138}\text{La} = 2.58 \times 10^{-12} \text{ yr}^{-1}$. $R_{\text{CHUR}}^{\text{Ce}}(0) = 0.022853$ is obtained as a value for $^{138}\text{Ce}/^{142}\text{Ce}$ in CHUR today (Fig. 3).

Several elements of REE in Isua gneisses were determined¹⁹. The lanthanum that was not analysed was estimated by extrapolation of Ce-Nd line on chondrite-normalized REE pattern.

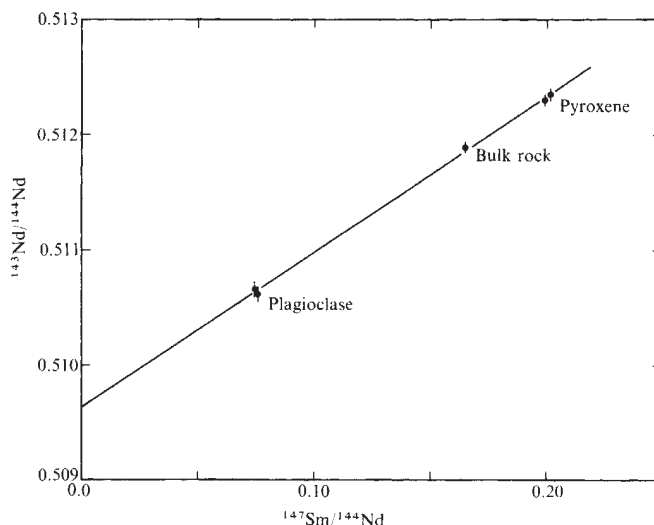


Fig. 2 Sm-Nd mineral isochron plot obtained from an aliquot of the same sample solution as that for La-Ce analysis. The isochron yields an age of $2,050 \pm 90$ (2 σ) Myr with an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50964 ± 0.00010 (2 σ) ($\lambda_{\alpha}^{147}\text{Sm} = 6.54 \times 10^{-12} \text{ yr}^{-1}$).

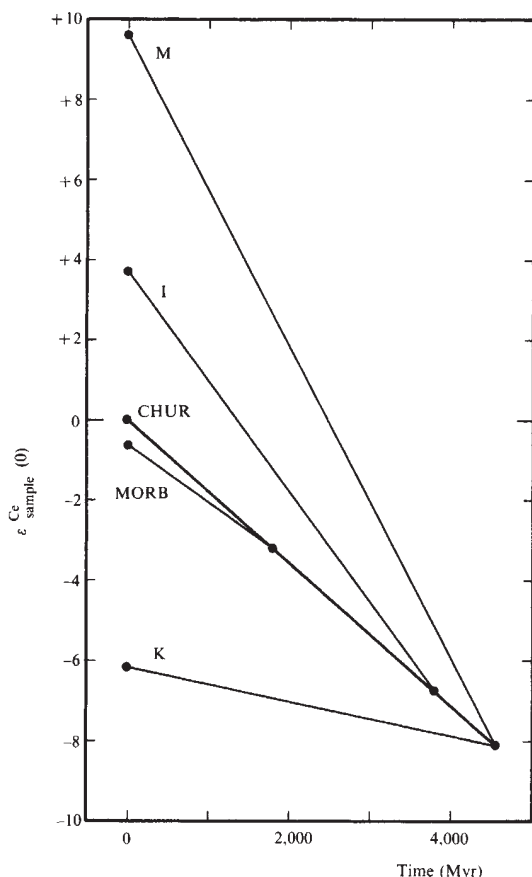


Fig. 3 Calculated $^{138}\text{Ce}/^{142}\text{Ce}$ growth curve; Melrose-b howardite (M), Isua gneiss (I), chondritic uniform reservoir (CHUR), mid-oceanic ridge basalt (MORB) and Khohar chondrite (K). Ordinate indicates $\epsilon_{\text{Ce}}(0)$ scale, that is relative to CHUR at present, 0.022853.

$(\text{La}/\text{Ce})_{\text{weight}} = 0.526$ was obtained for Isua granodiorite gneiss 155766. If we assume that the gneiss was formed from CHUR at 3,770 Myr ago²⁰, $\epsilon_{\text{Ce}}(0)$ of the gneiss is obtained to be +5.3 (Fig. 3). As discussed from Sm-Nd isotopic systematics by Masuda and Dohmoto²¹, if mid-oceanic ridge basalts (MORB) with $(\text{La}/\text{Ce})_{\text{weight}} = 0.318$ is assumed to be formed at 1,800 Myr ago, $\epsilon_{\text{Ce}}(0)$ of MORB is calculated to be -1.3 (Fig. 3). There should be a negative correlation between ϵ_{Ce} and ϵ_{Nd} .

For some meteorites with positive or negative Ce anomalies, such as Khohar (L3) and Melrose-b(howardite), larger variations of ^{138}Ce are expected. Khohar $(\text{La}/\text{Ce})_{\text{weight}} = 0.0911$ ²² and Melrose-b $(\text{La}/\text{Ce})_{\text{weight}} = 0.846$ ²³ are considered to have $\epsilon_{\text{Ce}}(0)$ of -12.3 and +19.3, respectively, under the assumption that those La/Ce variations were formed at 4,550 Myr ago (Fig. 3). A hibonite inclusion in Allende (HAL) has a La/Ce weight ratio higher than 103 (ref. 24). It will have $\epsilon_{\text{Ce}}(0)$ of more than 4,301.

We conclude that La-Ce isotopic system is a promising geochronometer and also will be a unique tool for isotope geochemistry. It is now essential to establish a reliable new value for the decay constant of ^{138}La .

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ESR dating of planktonic foraminifera

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Isotopic and palaeontological studies on Quaternary deep-sea sediment have provided relative but well-established time scales with ages mostly calibrated from the magnetic-polarity time scale. Absolute dating techniques such as U-series or K/Ar-dating have also been successful; however, difficulties exist such as elemental concentrations near the detection limit, or a lack of the necessary minerals. I report here on electron spin resonance (ESR) dating of marine cores, which utilizes the damage caused by natural radiation in planktonic foraminifera. An ESR signal grows linearly with the γ irradiation dose. It is found that the total radiation doses in the foraminifera increase with depth in two cores studied. The results demonstrate the viability of ESR dating for planktonic foraminifera up to 1 Myr in age.

The results presented here are for planktonic foraminifera of diameter 250-503 μm from deep-sea cores KH 73-4-7 and KN 73-4-8 (from the Melanesia Basin of the western equatorial Pacific Ocean, at $2^\circ 41' \text{N}$, $164^\circ 50' \text{E}$, water depth 4,160 m and $1^\circ 33' \text{S}$, $167^\circ 39' \text{E}$, water depth 4,000 m, respectively). ESR spectra of 150 mg samples were measured with an JES-ME2X ESR-spectrometer at room temperature. Core sediments were dated in units about 8 cm long. Artificial irradiation from a ^{60}Co source (dose rate of 10^4 rad h^{-1}) was performed at the Radiation Institute of Science and Industrial Research, Osaka University.

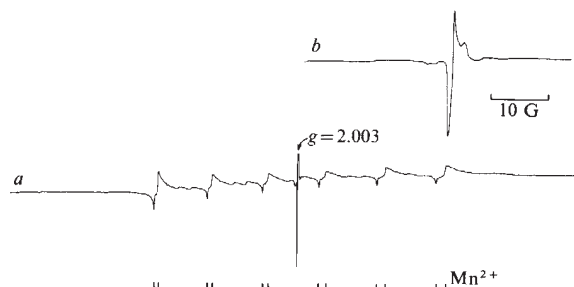


Fig. 1 Typical ESR spectrum of foraminifera. *a*, The signal with $g = 2.003$ is associated with the defects produced by natural radiation. The hyperfine line sextet of Mn^{2+} is also indicated. *b*, An expanded spectrum of the signal associated with the defects.

Table 1 Remarkable levels in two cores and ESR data

Levels	Ages (Myr)	KH 73-4-7				KH 73-4-8			
		Depths (cm)	Mean sedimentation rates (m Myr ⁻¹)	Mean annual doses (rad yr ⁻¹)	ESR ages (Myr)	Depths (cm)	Mean sedimentation rates (m Myr ⁻¹)	Mean annual doses (rad yr ⁻¹)	ESR ages (Myr)
Lower Olduvai boundary	1.87 ¹³	884 ⁶	— 4.0			884 [*]	— 0.6		
Upper Olduvai boundary	1.67 ¹³	805 ⁶	— 5.7			873 [*]	— 2.9		
Lower Jaramillo boundary	0.97 ¹³	407 ⁵	— 7.0	0.102	1.02	670 ⁵	— 6.9	0.113	0.93
Upper Jaramillo boundary	0.90 ¹³	358 ⁵	— 5.7	0.100	0.92	622 ⁵	— 5.2	0.116	0.88
Brunhes-Matuyama boundary	0.73 ¹³	261 ⁴	—	0.097	0.73 (Fixed)	533 ⁵	—	0.128	0.73 (Fixed)
Correspondence of palaeomagnetic intensity (lower)		145 [*]	5.3		0.50	280 [*]	13.8		0.52
<i>P. lacunosa</i> , Last appearance	0.474 ¹¹	125 ⁸	—	0.096	0.46	180 ⁸	—	0.107	0.42
Correspondence of palaeomagnetic intensity (upper)		40 [*]	2.6		0.30	110 [*]	3.8		0.34
<i>E. huxleyi</i> , First appearance	0.275 ¹¹								
Acme Zone	0.07 ¹⁴	15-22 [†]				71-80 [†]			0.31

The mean annual doses are estimated from the TDs shown in Fig. 3 and the reported ages for the levels. The ESR ages are estimated from the TDs shown in Fig. 3 and the mean annual dose at the Brunhes-Matuyama boundary; a constant annual dose assumed is arbitrarily set equal to the mean annual dose at the boundary for each core.

* Results on palaeomagnetic field intensity, which will be reported elsewhere.

† S. Nishida, personal communication.

Figure 1 shows a typical ESR derivative line of the foraminifera, with a predominant signal associated with the radicals created by natural radiation and the hyperfine line sextet of Mn²⁺.

The relative intensity of the radical signal is enhanced by exposure to ⁶⁰Co γ rays (Fig. 2). The total dose (TD) was determined by the additive dose method, in which the signal intensity of the samples is compared with that of identical samples which have also received a laboratory radiation dose. This method is used in thermoluminescence dating^{1,2} and ESR dating³, where the TD is frequently termed the AD (archaeological dose). To minimize errors caused by changes in instrument sensitivity and differences in sample weight, the signal intensity of the irradiated samples was measured relative to the ratio of the intensity of Mn²⁺ signals in the irradiated samples to the unirradiated ones, the Mn²⁺ signals were used as an internal standard. As the concentration of Mn²⁺ is high, the change in the signal intensity of Mn²⁺ by irradiation in this dose range is negligible. Growth of the signal intensity is almost linear up to ~250 krad. Maximum irradiation doses here are below 200 krad for samples which were expected to show high TD.

Figure 3 shows TDs obtained from the results shown in Fig. 2 as a function of core depth. The TDs seem to show an exponential increase towards a saturation level for each core. However, palaeomagnetic and biostratigraphical studies demonstrate that the mean sedimentation rate of the past 0.47 Myr is less for each core than in earlier times (Table 1): the Brunhes-Matuyama boundary, the Jaramillo event and the Olduvai event were observed for each core⁴⁻⁶. A microbiostratigraphical investigation of the cores studied by Takayanagi *et al.*⁷ showed a level of the last appearance of *Pseudoemiliania lacunosa*, which presumably occurred within isotope Stage 12 (~0.474 Myr)⁸. The small TDs at the uppermost portion are associated with the low sedimentation rate. Therefore the TDs should be considered to increase linearly with depth until the Jaramillo event as shown in Fig. 3. Note that the TDs estimated from the linear increase for the remarkable

levels are almost proportional to the reported ages in the levels in both cores, especially in KH 73-4-7. The mean annual dose is almost constant for each core as shown in Table 1.

The K-, U- and Th- contents within a deep-sea core^{9,10} seem to fluctuate rapidly with depth. The monotonic increase in the TD with depth may be interpreted as being the influence of variation in the contents smoothed out by penetration of the γ rays over 30 cm.

There are three possible causes of the extremely small TDs observed near the surface. First, changes can occur in the sedimentation rate due to dissolution of calcium carbonate, and deposition of really young sediment, or second, in the annual dose near the surface of the sea bottom. Wintle and Huntley¹¹ pointed out that the sediment within 30 cm below the sediment-water interface does not receive the full γ dose; therefore, it is possible that after a period of deposition at a constant rate, deposition may have ceased since ~0.2 Myr. Third, changes can occur in the annual dose due to the exponential decrease of the ²³⁰Th excess. In this case, the mean annual dose should also decrease but more gradually. The almost constant mean annual doses shown in Table 1 indicate little influence of the ²³⁰Th excess. A microbiostratigraphical study of the uppermost 1 m of the core (S. Nishida, personal communication) shows evidence of dissolution of calcium carbonate. Species which have thin and fine structures in a coccosphere were not observed and the species assemblage was simplified in both cores. A level of the first appearance of *Emiliania huxleyi*, which presumably occurred within isotope Stage 8 (~0.275 Myr)⁸, was observed by Nishida between 15 and 22 cm for KH 73-4-7. However, this portion may correspond to the beginning of the acme zone of the species, as the coccosphere is rather sensitive to dissolution. On the other hand, in KH 73-4-8 the level where the species first appeared was indicated between 71 and 80 cm, however, the species was not observed in portions of this core shallower than 31 cm. The disappearance of the species is probably due to dissolution and the fact that part of the acme zone could not be collected. We assume therefore that the small

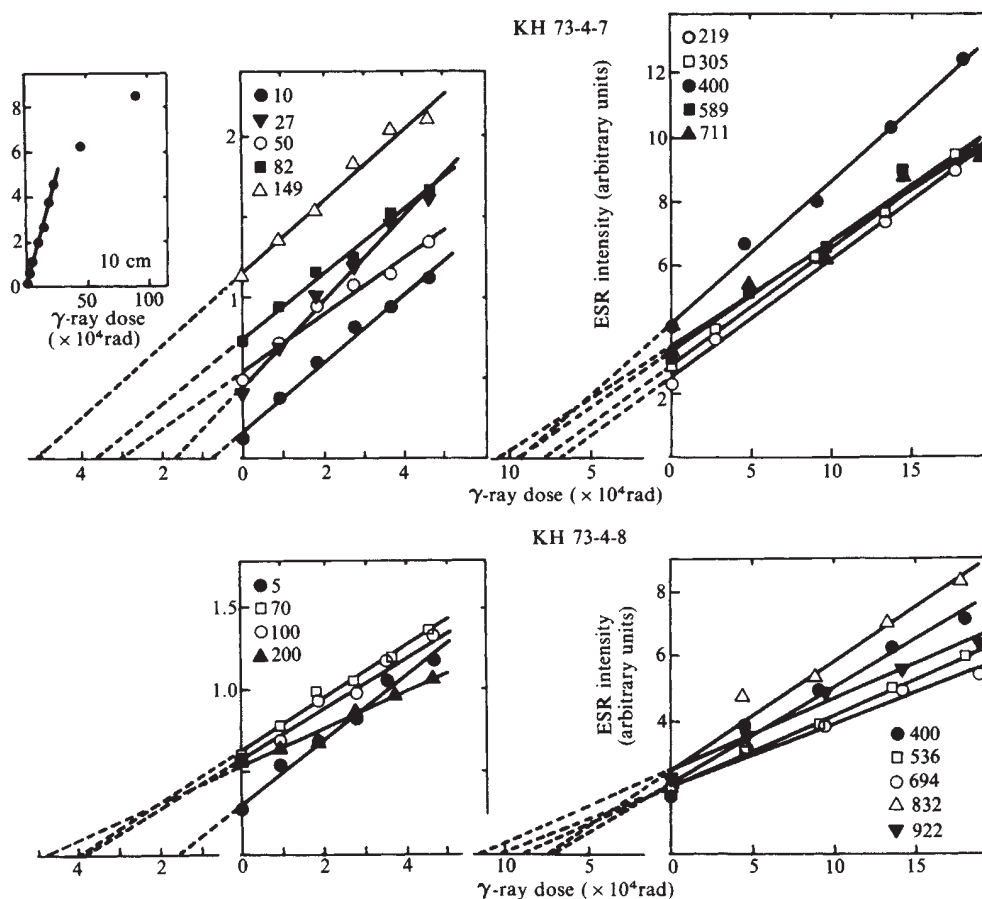


Fig. 2 Growth of the ESR signal of foraminifera from two cores with artificial γ irradiation. The numbers indicate the depths of each core.

TDs at the shallower depth are due to dissolution of calcium carbonate.

Samples taken from portions deeper than those corresponding to the Jaramillo event in both cores did not show any further linear increase of TDs with either depth or age, although the accuracy of TDs decreases. Wintle¹² reported that the mean lifetime of the 275°C TL peak of calcite was 390 Myr at 5°C, and that the anomalous fading, the effect of which could not be detected by mean lifetime estimation, had been observed for a sample of labradorite feldspar. As the tendency towards saturation was observed downward from the portion of similar ages in two cores with different depositional environments, it may be caused by the anomalous fading in calcite.

The ESR ages, determined on the basis of the ages of the Brunhes-Matuyama boundary, assuming a constant annual dose for each core, are compared in Table 1. Detailed palaeomagnetic investigation of these cores, in which variations in the palaeomagnetic field intensity were deduced from the ratio of natural remanent magnetization to the saturation isothermal remanent magnetization, showed correspondence between the curves of the ratio. The ESR ages of two distinctly corresponding depths are compared in Table 1. The results of the palaeomagnetic investigation will be described in detail elsewhere.

The disagreement between the reported ages and the ESR ages in KH 73-4-7 can be explained by dissolution to the order of 10% in the portion shallower than 2 m. On the other hand, the complicated changes in the sedimentation rate may be associated with the disagreement in KH 73-4-8. These changes lead to errors in the TDs estimated from linear increase. The TDs can be said to be almost proportional to the ages for a core with a constant sedimentation rate.

I conclude that ESR is useful for dating planktonic foraminifera up to 1-Myr old but to obtain detailed changes in the sedimentation rates, some problems must be resolved. More

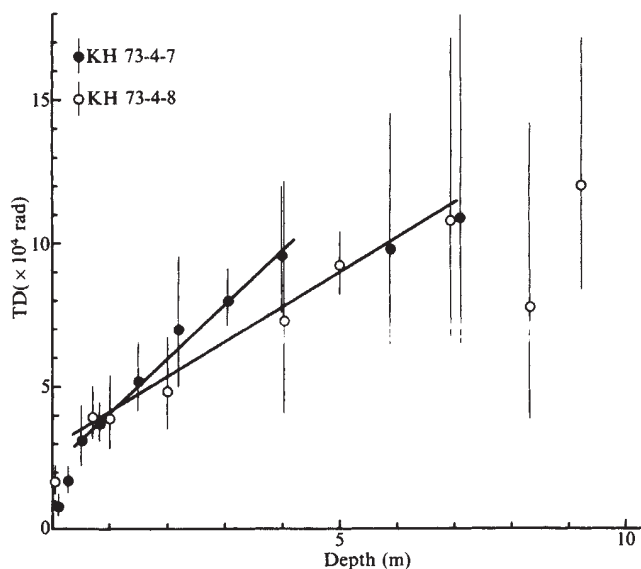


Fig. 3 Total dose (TD) plotted against depth for two cores. The bars represent the 90% confidence level in the TD measurement only.

accurate estimation of TD and evaluation of variations in the annual dose versus depth on the basis of the contents of radioactive elements and isotopes are necessary.

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Female mating preference is genetic

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The objection is often raised against sociobiological theory that different behavioural strategies have never been shown to be genetically determined. Yet if they are to evolve at all, the differences must be in part hereditary. Nobody would dispute that many aspects of behaviour—the song of *Drosophila* or birds, or the tendency to migrate, for example—would show genetic variation. But sociobiologists assert that different strategies of behaviour—the alternative courses of action that an animal might take—are also inherited and can thus be selected: strategies for example, such as whether to be a 'hawk' or a 'dove' in conflicts, or whether to choose one type of male because he may be a better mate than others. No doubt there are many aspects of behaviour that vary genetically and thus produce variations in the chances of success in conflicts or in finding mates. But does an animal prefer to follow one course of action rather than other alternative courses, and is its preference hereditary? This is the crucial question for sociobiology. As dogmatically as some sociobiologists have answered 'Yes', so have some population geneticists answered 'No'. Lewontin¹ called Dawkins' *The Selfish Gene*² a caricature of Darwinism for assuming that all behaviour may be inherited and natural selection can therefore do anything. These extreme views persist for lack of experimental evidence. We show here that female mating preference can be substantially increased in successive generations by artificial selection. It must therefore have a genetic component.

In *The Descent of Man*, Darwin³ invoked female preference as one of the two selective mechanisms for the evolution of male characteristics of sexual display. The existence of female preference was a premise of Darwin's theory. Fisher⁴ explained its existence as the outcome of an evolutionary process in which both preference and preferred character evolve together. O'Donald^{5,6} and Lande⁷ analysed models of female preferences that support Fisher's theory. Models of preferential mating have been fitted to observations on the relative frequencies of matings of different genotypes⁶, but experiments on the sexual selection of preference have never been carried out over a number of generations.

Preferential mating may be involved in the maintenance of the colour polymorphism of the two-spot ladybird, *Adalia bipunctata*^{8,9}. Majerus et al.¹⁰ carried out a series of mating choice experiments on melanistic (*quadrifasciata*) and non-melanistic (*typica*) ladybirds collected at Keele in Staffordshire. *Quadrifasciata* is inherited as a unifactorial dominant to *typica*¹¹. The results give a good fit to a model of mixed sexual selection and assortative mating for dominant and recessive traits¹². The maximum likelihood estimates generated by the model show a strong and very significant non-assorting preference, γ , for *quadrifasciata* males. The assorting preference,

c , for these males was appreciable, but not formally significant. Maximum likelihood estimates of assorting and non-assorting preferences for *typica* were very close to zero¹⁰. Observations of courting pairs indicated that female choice determined the frequencies of the different matings. No male competition for mates has been seen to occur¹⁰.

Following these results, it was obviously imperative to test whether selection will increase the level of female preference in successive generations, and also to confirm experimentally that female choice determines the frequencies of the matings of the different male phenotypes. A sample of ladybirds from the Keele stock was allowed to mate in a population cage. The sample consisted of the following phenotypes: 112♂♂ + 112♀♀ *typica*, 48♂♂ + 48♀♀ *quadrifasciata*. This 7:3 ratio of *typica*:*quadrifasciata* was chosen because it gives good estimates of the mating preference at the level at which it occurs in the Keele stock. Our experimental regime has been described elsewhere¹⁰. We recorded the phenotypes of individuals in copuli by continuously observing the cage during a fixed period of 16 h of light per day. We removed the mating pairs of ladybirds. As soon as pairs had separated, we replaced the males in the cage. We placed those females that had mated with *quadrifasciata* males in a new population cage to lay the eggs for the next generation of the selected line. Females which mated with *typica* males were discarded. We repeated this procedure for three subsequent generations, restoring the 7:3 phenotypic ratios at the start of each generation. We also set up a control line, maintained in the same way as the selected line, except that all mating females, regardless of the phenotypes of their mates, laid the eggs of the next generation. The results from both the selected and control lines are given in Table 1. If the deviation from random mating shows an overall preference towards *quadrifasciata* males, then matings take place at the frequencies $\gamma + v(1 - \gamma)$ for *quadrifasciata* males, and $(1 - v)(1 - \gamma)$ for *typica* males, where v is the overall frequency of *quadrifasciata* in the cage and γ the proportion of females expressing a preference for them. Even if the preference is expressed in some partial or frequency-dependent manner, the constancy of v in all experiments ensures that γ will be a constant fraction of the total proportion of females with a preference; hence, γ will always measure the relative magnitude of the preference^{6,13}. If n_Q and n_T are the observed numbers of the matings with each phenotype of male, the level of preference is estimated by

$$\hat{\gamma} = \frac{n_Q(1 - v) - n_T v}{n(1 - v)}$$

where $n = n_Q + n_T$. This maximum likelihood estimate has been derived from a model with no preference for *typica*. Table 1 shows the estimates of preference against the data from which they were obtained. In generation 3, we also set up direct tests of female choice in mating chambers, using progeny from the previous generation and equal phenotypic ratios. In these experiments, each mating pair is immediately replaced by fresh individuals with the same phenotypes as those that had mated. We tested female choice by similar experiments on the original unselected stock. Table 2 shows the results of these mating test experiments.

To differentiate conclusively between male competition and female choice, we set up three further population cage experiments. A sample consisting of males from the progeny of the fourth generation of the selected line and females from the unselected Keele stock were allowed to mate in a population cage. In a second cage, females from the fourth generation of the selected line were allowed to mate with males from the unselected Keele stock. In a third cage, as a control, males and females both taken from the unselected Keele stock were allowed to mate. In all three experiments, 7:3 ratios of *typica*:*quadrifasciata* phenotypes were maintained in the samples. Table 3 gives the frequencies of matings, estimates of preference and statistical analysis.

Table 1 Numbers of ladybirds in population cages in successive generations and estimates of female preference

Selected line		Total nos in cage		Nos of matings between different phenotypes			Estimates of preference for Q $\hat{\gamma} \pm \sqrt{\text{var}(\hat{\gamma})}$
Generation	N_T	N_Q	Q×Q	Q×T	T×Q	T×T	
1	224	96	21	31	24	46	0.1803 ± 0.0640
2	112	48	13	18	9	14	0.3915 ± 0.0961
3	84	36	12	14	6	10	0.4558 ± 0.1070
4	140	60	18	21	5	12	0.5663 ± 0.0878
Control line							
1	98	42	13	15	8	21	0.2732 ± 0.0946
2	70	30	7	11	7	12	0.2664 ± 0.1174
3	112	48	10	18	10	18	0.2857 ± 0.0955
4	112	48	11	22	13	28	0.2085 ± 0.0825
Analysis of χ^2							
Component of variation				Values of χ^2		d.fs	Values of P
Between selected and unselected groups				12.5103		1	0.000405
Within selected populations				1.6837		2	0.431
Within unselected populations				1.3000		4	0.861

N_T and N_Q refer to the total number of *typica* (T) and *quadrimaculata* (Q) ladybirds of a given generation that were placed in a population cage for mating. They consisted of equal numbers of males and females. The male phenotype is shown first in the type of mating: Q×T is a mating of Q♂×T♀; T×Q is a mating of T♂×Q♀. The males were replaced in the cage after they had mated. The preferred males often mated more than once during the 5 or 6 days of observation of a cage. Females were not replaced after mating. If observations had been continued until all females had mated, an exact 7:3 ratio of T:Q females would necessarily have been found to have mated. However, after 6 days, 90% of Q and 60% of T females had mated. Comparing numbers of mated and unmated females among Q and T females in the selected line, we obtain $\chi^2_3 = 1.4962$, showing no change in mating propensities of Q and T females with increasing preference over generations. The Q and T females show similar levels of preference, but the T females are slower to mate. 'd.fs' in the analysis of χ^2 means the numbers of degrees of freedom.

Table 1 shows that the proportion of females preferring *quadrimaculata* males increased significantly in the selected line but not in the control line. Although obviously decisive, these results present a problem of statistical analysis. A linear increase in preference is not to be expected because the selection differential declines as more females exercise the preference: sexual selection by female choice is necessarily frequency-dependent^{6,14}. We should expect that the rate of increase of preference would fall rapidly in successive generations; how rapidly would depend on the genetics of preference which is unknown. A simple linear regression of the increase of γ would be an inappropriate model to fit to the data. The data of both selected and unselected lines form a 2×8 table of numbers of matings of *quadrimaculata* and *typica* males. Generations 2, 3 and 4 of the selected line represent generations after females have been selected for increased preference. These generations can be tested against the unselected generations. Of course, this test ignores the increase within the selected line. It represents a conservative test which should not overestimate the true level of significance.

The data also show that the response of the selected line differs significantly from that of the control line. This is the crucial test. In the absence of an appropriate regression model, a conservative test of the difference in the responses can be constructed by comparing the differences between the first and fourth generations in the selected and control lines. We expect to find a response over the four generations in the selected line but not in the control line. Thus, we ask: do the selected and control lines differ in their responses? The data form a 2³ table as follows:

	Selected line		Control line	
	Q♂♂	T♂♂	Q♂♂	T♂♂
Generation 1	52	70	28	29
Generation 4	39	17	33	41

The difference in Q:T ratio between selected and control lines differs significantly between generations. For this three-factor interaction (phenotypes×lines×generations): $\chi^2_1 = 6.2678$, $P = 0.0123$. Because we have omitted the data of generations 2

and 3, this is a very conservative test which must underestimate the true level of significance in the total data. The difference between selected and control lines in generation 4 is tested by $\chi^2_1 = 8.0939$ (or 7.1120 with Yates' correction), $P = 0.00444$ (or 0.00766 for χ^2 with Yates' correction). The preference has been raised very significantly in the selected line but not in the control line. As shown in Table 2, the difference between selected and control lines in generation 3 is nearly significant in spite of the very small numbers of matings observed for this generation in the selected and control lines. By Fisher's combination of probabilities test applied to the results of generation 3 and 4, we obtain $\chi^2_4 = 14.9761$, $P = 0.00475$, giving the overall significance of the difference between the lines.

Table 2 Numbers of matings of ladybirds in mating choice experiments and estimates of female preference

Mating tests on original unselected stock				
Nos of matings between different phenotypes				Estimate of preference for Q $\hat{\gamma} \pm \sqrt{\text{var}(\hat{\gamma})}$
Q×Q	Q×T	T×Q	T×T	
72	67	41	46	0.2301 ± 0.0522
Mating tests on generation 3 of selected line				
15	15	6	4	0.5 ± 0.1369
Overall estimates of preference for generation 3				
Preference in selected line in generation 3 estimated from data of mating tests and population cage experiments:				$\hat{\gamma} = 0.4721 \pm 0.0846$
Preference in control line in generation 3:				$\hat{\gamma} = 0.2857 \pm 0.0955$
Test of significance of difference in estimates for control and selected lines:				$\chi^2_1 = 3.2120$; $P = 0.0731$

In these mating test experiments, five males and five females of each phenotype are placed in a mating chamber and kept under continuous observation. When a mating takes place, the mating pair are removed and fresh individuals of the same phenotypes are replaced. Both male and female phenotypes are replaced. A strict 5:5 ratio of Q:T males and females is thus maintained in the mating chamber.

Table 3 Mating tests between the selected line and original Keele stock

Nos of matings and estimates of preferences		Nos of matings between different phenotypes			Estimates of preference for Q $\hat{\gamma} \pm \sqrt{\text{var}(\hat{\gamma})}$
Stocks and sexes used in matings	Q × Q	Q × T	T × Q	T × T	
Selected ♂♂ × Keele ♀♀	11	28	17	34	0.1905 ± 0.0746
Selected ♀♀ × Keele ♂♂	16	28	6	15	0.5385 ± 0.0829
Keele ♂♂ × Keele ♀♀	18	35	21	42	0.2241 ± 0.0661
Analysis of χ^2					
Component of variation	Value of χ^2			d.fs	Value of P
Selected males compared with Keele males	0.1126			1	0.737
Selected females compared with Keele females	10.4844			1	0.00120

The selected males and females used in these tests were taken from the progeny of the fourth generation of the selected line. No further selection had been imposed after the fourth generation. The level of preference among the progeny of the fourth generation should be the same as that in the fourth generation. The selected males and females were tested in matings with progeny taken from the corresponding generation of the original Keele stock. Population cages were set up with 7:3 ratios of T:Q. They were kept under continuous observation; mating pairs were counted giving the numbers shown in the table.

The response to selection that we have demonstrated has taken place in the females' mating preference for *quadrinaculata* males. As shown in Table 3, when selected females are mated with males from the original stock, the level of preference is characteristic of the selected line; when selected males are mated with females from the original stock, the level of preference is characteristic of the original stock. Female mating preference has thus been shown to have a large genetic component in its determination. We believe that these experiments provide the first formal proof that a female mating strategy is genetically determined.

This proof is fundamental for theories of the evolution of female preference⁴⁻⁷. It shows that female choice of mate can evolve: mate choice will be selected if, as in our experiment, females making the choice contribute a relatively greater proportion of offspring to future generations. In a natural population, they will do so if the males they choose to mate with possess a selective advantage. As the female preference contributes some of this selective advantage, it partly selects itself. This gives rise to what Fisher⁴ called the 'runaway process of sexual selection'. Initially, while preference genes are rare—they may perhaps have arisen by mutation—this process will be extremely slow, requiring many thousands of generations to get started⁵. Realistically, male competition or natural selection must provide the initial advantage. The preference will then be selected in the course of the selection of the preferred male character. The dynamics of this selection is very complex and depends crucially on the genetics of the preference⁵. Initially, as a gene for the preferred character spreads through a population, a preference gene may be expected to show a three- to fivefold increase in frequency. As the preference becomes more common, it adds to the preferred males' advantage; but the preference gene itself shows progressively smaller increases in frequency as it becomes more common. The process eventually stops when the male character has developed to the point at which it has become so disadvantageous in natural selection that this disadvantage balances the sexual selection.

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Phenomenal coherence of moving visual patterns

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When a moving grating is viewed through an aperture, only motion orthogonal to its bars is visible, as motion parallel to the bars causes no change in the stimulus. Because there is a family of physical motions of various directions and speeds that appear identical, the motion of the grating is ambiguous. In contrast, when two crossed moving gratings are superimposed, the resulting plaid pattern usually moves unambiguously and predictably. In certain cases, however, two gratings do not combine into a single coherent percept, but appear to slide across one another. We have studied the conditions under which coherence does and does not occur, and we report here that it depends on the relative contrasts, spatial frequencies and directions of motion of the gratings. These effects may reveal the previously unstudied properties of a higher order stage of motion analysis.

Figure 1a illustrates the problem of ambiguity^{1,2}. The grating moves behind a circular aperture. Any of the physical motions indicated by the arrows will appear the same when viewed through the aperture. The situation can be depicted graphically in 'velocity space', a space in which each vector represents a velocity: the length of a vector corresponds to speed, and its angle corresponds to direction. As shown in Fig. 1a, the motion of a single grating is consistent with a family of motions that lies along a line in velocity space; this line is parallel to the grating's bars and orthogonal to the vector representing its 'primary' motion. If two moving gratings are superimposed, the resulting 'plaid' pattern moves with a speed and direction which can be exactly predicted from the velocity space construction: the two loci of possible motions intersect at a single point, corresponding to the motion of the coherent pattern (Fig. 1b). This solution to the 'aperture problem' is similar to that proposed (in a rather different context) by Fennema and Thompson³. It has advantages over that advanced by Marr and Ullman⁴, which can only infer a range of possible directions from the limited information provided by a pair of gratings.

The velocity space combination rule is also different from a vector sum or vector average. In Fig. 1c, two gratings move downward and rightward with different speeds and directions.

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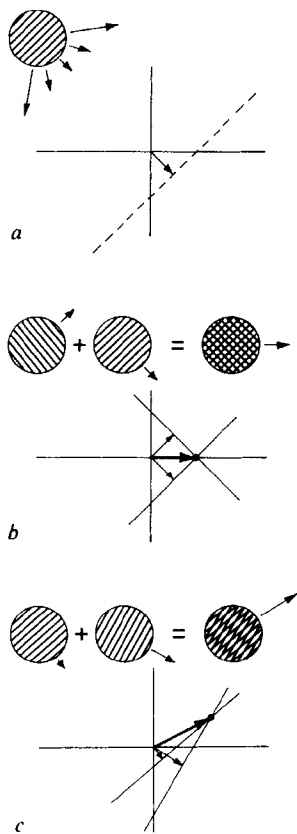


Fig. 1 The velocity-space representation of some moving patterns. In each panel, a vector represents a motion in a direction given by the vector's angle at a speed given by the vector's length. *a*, A single grating moves behind an aperture. The broken line indicates the locus of velocities compatible with the motion of the grating. *b*, A plaid composed of two orthogonal gratings moving at the same speed. The lines give the possible motions of each grating alone. Their intersection is the only shared motion, and corresponds to what is seen. *c*, A plaid composed of two oblique gratings, one moving slowly and the other more rapidly. Both gratings move down and to the right, but the pattern moves up and to the right.

A vector sum or average would predict a pattern motion that was also downward and rightward, but the velocity space solution is a motion upward and rightward, the motion that observers report when the gratings are seen to cohere⁵. Of course, nothing requires that coherent motion be seen, and instead of a single rigidly moving plaid, the two gratings are sometimes seen sliding 'incoherently' over each other. We have studied the stimulus conditions under which coherence occurs, in order to characterize the mechanisms that underlie the coherent percept and resolve the ambiguity of one-dimensional motion.

Observers viewed a circular cathode ray tube display subtending 5°; the luminance of the display was constant on average, but was modulated by signals from a PDP11 computer to produce a superimposed pair of sinusoidal gratings that could each be varied in orientation, direction and speed of motion, contrast and spatial frequency. Observers were strictly instructed not to make judgements unless their eyes were steadily fixated on a small black circle in the centre of the display. Subjects under this kind of instruction are capable of stable and reliable fixation⁶. After each 1.5-s exposure, the observer indicated whether the pattern appeared 'coherent' or 'incoherent'.

Contrast strongly affects coherence, even when both gratings are easily visible. Figure 2*a* shows a psychometric function for detection and a similar function for coherence, when the contrast of one of the gratings in a plaid was varied (the other grating's contrast was fixed at 0.3). The gratings were of the same spatial frequency, and moved at an angle of 135° to one

another. Open symbols show the probability of detecting the low-contrast grating in the presence of its high-contrast mate. Filled symbols show the probability of a coherent percept. Both detection and coherence become more likely as contrast increases, but reliable coherence only occurs at contrasts where both gratings are clearly visible.

In further experiments, we used this contrast dependence to measure the relative tendency of different pairs of gratings to cohere. We fixed the contrast of one grating at 0.3, and used a staircase procedure to vary the contrast of the other grating until the observer saw coherent motion on half the trials. The gratings' speed, orientation and spatial frequency all affected the strength of coherence. Coherence decreased as the speed of the component gratings increased, as the angle between their 'primary' directions increased, and as the difference between their spatial frequencies increased.

Figure 2*b* shows the effect of relative spatial frequency on coherence. Filled symbols illustrate a case in which the spatial frequency of the high-contrast grating was fixed at 1.2 cycles deg⁻¹, while the spatial frequency and contrast of the second grating were varied; open symbols illustrate a case where the spatial frequency of the first grating was fixed at 2.2 cycles deg⁻¹. As the frequencies of the two gratings were made different, the tendency to cohere was reduced, and the contrast needed for coherence was increased. This spatial frequency dependence suggests that coherent motion, like many other visual properties, is analysed by mechanisms that are selective for spatial frequency.

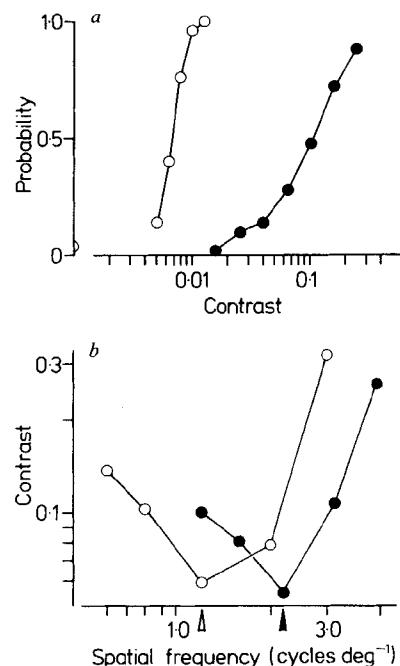


Fig. 2 Two parameters influencing perceptual coherence. *a*, The influence of contrast on the detectability and coherence of two crossed sinusoidal gratings. One grating was fixed in contrast at 0.3. The other was of variable contrast, and moved at an angle of 135° to the first; both had a spatial frequency of 1.6 cycles deg⁻¹, and moved at 3 deg s⁻¹. Filled symbols show the probability that the observer judged the two gratings to be 'coherent'; open symbols show the probability that the observer detected the presence of the lower contrast grating. The half-circle on the ordinate shows the probability that the observer judged the second grating to be present when its contrast was zero—the 'false-positive rate' for detection; the false-positive rate for coherence judgments was zero. Subject, E.H.A. *b*, The influence of spatial frequency on coherence. The standard grating again had a contrast of 0.3 and moved at 3 deg s⁻¹. The test gratings moved at an angle of 135° to the standard, also at 3 deg s⁻¹. Open symbols show the results for a range of test spatial frequencies when the standard grating had a spatial frequency of 1.2 cycles deg⁻¹ (open arrow). Filled symbols show the results when the standard grating had a spatial frequency of 2.2 cycles deg⁻¹ (filled arrow). Subject, P.A.

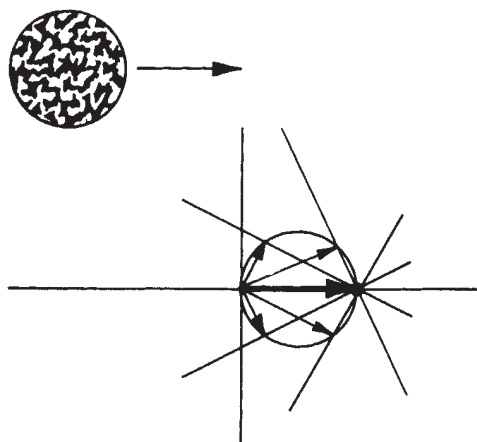


Fig. 3 The velocity-space representation of a random-texture field moving to the right. The field contains components of all orientations, and the primary motion vector for each ends on the circle passing through the origin; the common motion 'implied' is the rightward motion given by the bold arrow. This circular locus of primary motions represents all the motions that can exist in a single rightward-moving pattern. As such, the locus could represent the preferences of a family of primary motion analysers whose outputs are combined to signal coherent two-dimensional motion (see text).

There are two kinds of model that might account for our results. The first relies on the presence in our patterns of localizable blob-like features where the peaks and troughs of the crossed gratings intersect. Some process based on direction-selective elements not selective for orientation might detect and signal the motion of these 'blobs', which is of course identical to the motion of the pattern as a whole. We have evidence to suggest that such a scheme is incorrect. We superimposed one-dimensional dynamic random visual noise (a rapidly changing pattern of random-width lines)⁷ on our test patterns. A non-oriented mechanism that responds directly to the motion of blobs should be most affected by noise oriented at right angles to the coherent motion. But a mechanism that combines the outputs of oriented channels should be most affected when those channels are most affected—when the noise is oriented at right angles to the primary motion of one or the other of the component gratings. Our results support the orientation-based model. Coherence is strongly reduced when the noise mask is perpendicular to a component's motion (and thus parallel to the component's orientation), but is almost unaffected when the mask is perpendicular to the coherent motion of the pattern. This suggests that some orientation-selective process must precede the analysis of coherent motion, and our second model incorporates this feature.

There is abundant evidence that visual analysing mechanisms selective for orientation and spatial frequency exist at a relatively early stage in the visual pathway⁸⁻¹². It is clear that such mechanisms are often sensitive to the direction of motion of one-dimensional contours^{8,9,13-15}, but they also seem unable to signal the true direction of motion of two-dimensional patterns¹⁶⁻¹⁹. The perceptual coherence of two gratings into a single moving plaid may therefore represent the action of an additional stage of visual processing beyond those usually considered to be involved in analysing motion. At this second stage, the outputs of several 'one-dimensional' motion analysers would be combined. Coherence phenomena may offer an approach to studying the properties of this postulated second-stage motion analyser.

Figure 3 shows how the velocity space construct helps to assign motion to more complex patterns, such as a horizontally moving random-texture field. The texture contains components of many orientations; their motions are represented in velocity space by the arrows in Fig. 3. As the speed of each component is proportional to the cosine of the angle between its direction and horizontal, the vectors all end on a circle that passes through

the origin. Note also that the motion 'families' associated with the various components all pass through the common point corresponding to the horizontally directed pattern motion.

A given vector in Fig. 3 could represent the preference of a one-dimensional motion detector, and the circle might represent the 'receptive field' in velocity space of a second-stage motion analyser. If it combined the outputs of one-dimensional analysers corresponding to points on the circle by an operation akin to a logical 'and', the second-stage analyser would respond only to a particular direction of motion of the two-dimensional stimulus. Our results on perceptual coherence might reflect the relative strengths of the different inputs to such a motion analyser. They would suggest that this analyser computes the weighted combination of signals arising from a collection of one-dimensional motion analysers having different velocity preferences but similar spatial frequency preferences.

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Prenatal misrouting of the retinogeniculate pathway in Siamese cats

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In the mammalian visual system, retinal ganglion cells send their axons to several distinct structures in the brain. One of these, the lateral geniculate nucleus (LGN), receives a substantial projection from both eyes in higher mammals such as the cat¹⁻³. How does this pattern of input arise during development? One possibility is that retinal ganglion cell axons project from the very beginning to the appropriate side of the brain. Another is that the initial projection may contain many inappropriate connections which are eliminated after the axons reach their target, the LGN. We thought the Siamese cat might provide an opportunity to study these alternatives. Guillery and others⁴⁻⁷ have shown that in adult Siamese cats, the LGN receives a disproportionately large input from the contralateral eye and a correspondingly diminished input from the ipsilateral eye due to a genetic mutation at the *albino* locus. We report here that this abnormal pattern of input is present from the earliest stages of development of the retinogeniculate projection and arises as a consequence of the misrouting of fibres at the optic chiasm. These findings suggest that during normal development, growing optic fibres can be directed to the appropriate side of the brain.

In normal adult cats the retinal ganglion cells within each eye are sharply divided into two halves by virtue of their axonal projections to the LGN^{8,9}. Figure 1a shows that axons from retinal ganglion cells in the nasal half of each eye cross in the optic chiasm and terminate in layers A, C and C2 of the contralateral LGN. Axons from retinal ganglion cells in the temporal half of each eye do not cross but project instead to the ipsilateral LGN where they terminate in the alternate A1 and C1 layers^{1,10}.

In contrast, in adult Siamese cats the nasotemporal division is abnormal, (see Fig. 1b). In these animals the contralateral projection to the LGN consists of axons from retinal ganglion cells in the nasal retina, as usual, plus an abnormal component from the temporal retina which projects to portions of layers A1 (abA1) and C1. A reduced number of the remaining retinal ganglion cells in the temporal half of the retina send axons to the ipsilateral LGN, where they are confined to several small patches in layers A1 (mnA1 and lnA1) and C1^{6,7,11,12}.

We wished to determine how the abnormal Siamese pattern of the retinogeniculate projection arises during development. Therefore, we labelled the projection in 26 Siamese and 43 normal newborn and fetal cats of known gestational age by using the axonal transport of intra-ocularly injected horseradish peroxidase (HRP) or ³H-leucine. (Siamese fetuses were obtained from breedings within our own pure-bred Siamese colony.) The cat's gestation period averages 65 days, with the first day of breeding designated as embryonic day 0 (E0) and birth as postnatal day 0 (P0). Prenatal eye injections were performed using the fetal surgery techniques of Rakic¹³ modified for the cat¹⁴. In most cases both eyes were injected with different labels. Animals were allowed to survive for 24 h, then removed by caesarean section and processed for HRP histochemistry^{14,15} and/or autoradiography⁷. Complete and even labelling of the retinas in all the animals studied here was verified by examining the retinas (autoradiographically or histochemically) and by inspecting the pattern of the retinal projections to the LGN and superior colliculus. At each age the labelling pattern was considered to reflect accurately the retinogeniculate projection only when similar results were obtained from three or more injections.

By birth the pattern of axonal projection from retina to LGN as revealed autoradiographically or histochemically is adult-like, both in Siamese and normal cats—see top of Fig. 2, which shows a matched series of darkfield photographs of peroxidase- or radioactively-labelled sections through the LGNs of progressively younger normal and Siamese cats. At postnatal day 2, comparison of Fig. 2a (normal) and f (Siamese) demonstrates the drastically reduced ipsilateral and correspondingly increased contralateral projections that are typical of the retinogeniculate projection in adult Siamese cats. Compare also Fig. 2f with Fig. 1b—what remains of the ipsilateral projection in Fig. 2f is confined to several small labelled patches.

We next made a prenatal study. The left-hand side of Fig. 2 shows the general time course and pattern of development of the retinogeniculate projection in a series of normal cat fetuses. By E32, a clear projection from the retina to the lateral margin of the thalamus is present bilaterally, with that from the contralateral eye being more extensive than that from the ipsilateral eye (Fig. 2e). Note that at this age the label is largely restricted to the optic tracts, particularly ipsilaterally. At older ages (E35, E40; Fig. 2d, c, respectively), the ipsilateral and contralateral projections increase, and occupy progressively more medial territory within the LGN. By E54 (Fig. 2b), afferents from the two eyes are in the process of segregating from each other to form the set of sharply defined layers present at birth (Fig. 2a). (For fuller accounts of normal development, see refs 14, 16.)

The overall developmental pattern of the retinogeniculate projection in Siamese cats shown on the right-hand side of Fig. 2 differs from that of normal animals in one important respect; at each embryonic age there is a striking reduction in the size of the ipsilateral projection and a relative increase on the contralateral side. This size disparity is most apparent at E51

(Fig. 2g) when, as in normal animals, the retinogeniculate afferents from the two eyes are in the process of segregating from each other. In the Siamese fetus, however, the result is the appearance of the characteristic abnormal adult-like pattern (compare Fig. 2f and g). Earlier, at E46 (not shown), the projection pattern is similar to that seen at E51 in that the projection to the ipsilateral LGN remains significantly smaller in size and extent than that to the contralateral side. At E41, when the contralateral projection to the LGN is already well established, only a small contingent of ipsilateral fibres have invaded the posterior pole (Fig. 2h, arrow). Most of the remaining ipsilateral label is confined to the optic tract and brachium of the superior colliculus. Before E41, ipsilateral label is almost entirely restricted to the tract in the Siamese fetuses. This is the case at E36 (Fig. 2i), when the LGN is invaded normally by the first ipsilateral retinal fibres (Fig. 2d, arrow). Indeed, as early as E32, when the retinogeniculate afferents initially arrive within the vicinity of the LGN anlage, but in normal animals are still confined largely within the optic tract (Fig. 2e), the ipsilateral projection is absent from the Siamese fetus (Fig. 2j).

These results indicate that the LGN receives a diminished ipsilateral retinal projection throughout fetal development in Siamese cats. It is worth noting here that in the development of retinogeniculate connections in *albino* rats, Lund and co-workers¹⁷ found that the diminished ipsilateral projection emerges only secondarily, largely due to a massive elimination of connections once they had reached the LGN. Such differences between the two species may simply reflect differences in the action of the mutant gene. Alternatively, they may represent significant differences in the mechanisms underlying normal development—mechanisms that in higher mammals, but not in rodents, operate to produce a large and precise ipsilateral projection.

At least two distinctly different events could account for this reduction in the size of the ipsilateral projection within the

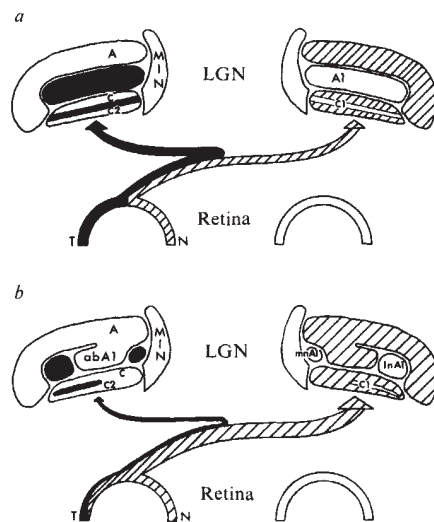


Fig. 1 A diagram of the retinogeniculate projection in normal (a) and Siamese (b) adult cats. Only connections between the left eye and the LGN are shown here for simplicity. a, Normally, axons from retinal ganglion cells in the nasal half of each retina (hatched area labelled N) cross at the chiasm and project to layers A, C and C2 of the contralateral LGN. Those in the temporal half (black area labelled T) project ipsilaterally to the alternate A1 and C1 layers. b, In the Siamese cat, the LGN receives an abnormally large projection from the contralateral retina (right LGN: hatched area), consisting of additional fibres from the temporal retina (hatched area) that innervate portions of layers A1 (abA1) and C1. There is a correspondingly reduced projection from the ipsilateral retina (left LGN: black areas) that terminates in several small patches medially (mnA1) and laterally (lnA1) in layer A1, and in layer C1. The projection to the medial interlaminar nucleus (MIN) is similarly affected in Siamese cats but is not shown here.

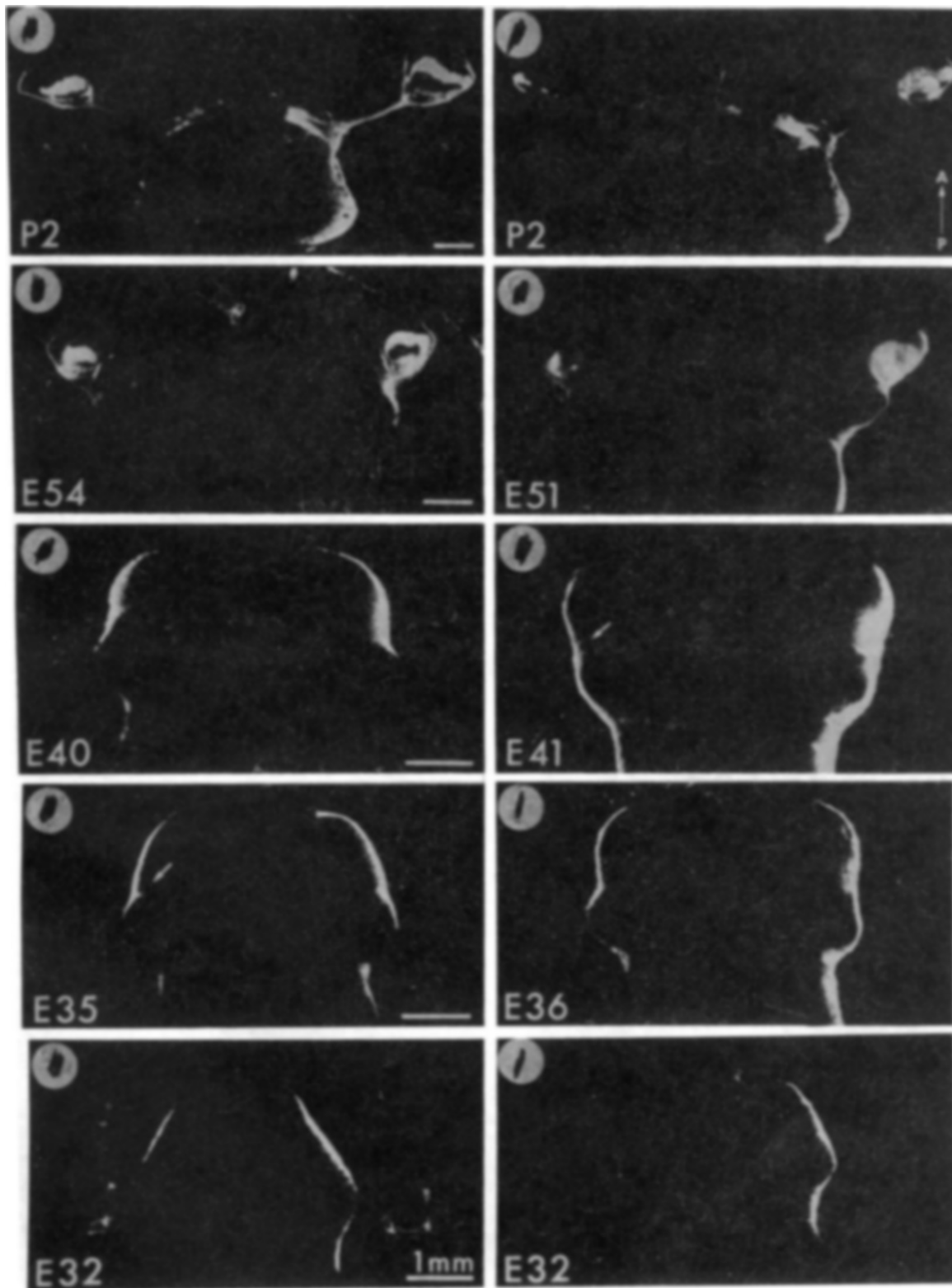


Fig. 2 Two series of darkfield photographs of HRP- or radioactively-labelled retinal afferents in horizontal sections through the lateral geniculate nuclei of normal and Siamese cats to show the progressive development of the retinogeniculate pathway. Postnatal (P) and embryonic (E) ages are indicated, as are the sides contralateral (contra.) and ipsilateral (ipsi.) to the injected eye. Due to the cat's extended gestation period (65 days), reasonable comparisons can be made between normal and Siamese fetuses differing by up to a few days in embryonic age. For each Siamese case, the section shown contained the largest amount of ipsilateral label seen. Label in the top half of each photo is contained within the LGNs and optic tracts. Label in the bottom half lies within the pretectum and superior colliculus. Arrows in *d* and *h* indicate label within the most posterior pole of the LGN. In *f*: A represents anterior, P posterior. The calibration bar (1 mm) for each pair of ages is shown in the left-hand (normal) series. See text for further details.

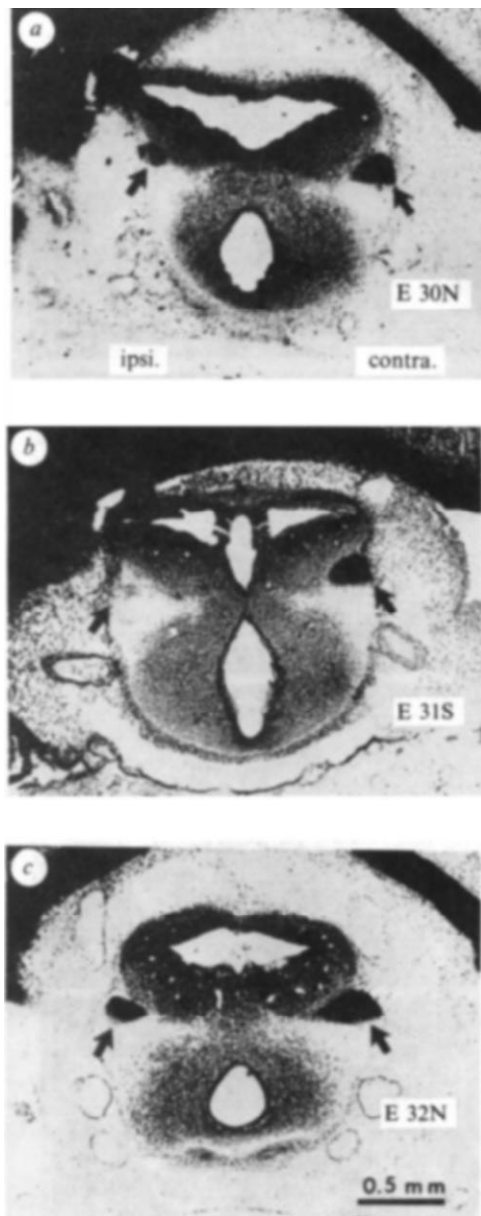


Fig. 3 Brightfield autoradiographs of horizontal sections through the optic tracts immediately adjacent to the chiasm to compare the size of the ipsilateral (ipsi.) and contralateral (contra.) retinofugal projection in three fetuses that received intra-ocular injections of ^3H -amino acids. Arrows indicate label (black) in the optic tracts. *a*, Normal E30 fetus; *b*, Siamese E31 fetus; *c*, Normal E32 fetus. The sections were counterstained with cresyl violet. Anterior is up, posterior down. Calibration bar (0.5 mm) is for *a-c*.

LGN of Siamese cats. It might arise either as a direct consequence of axonal misrouting at the optic chiasm, as originally proposed by Guillery^{3,4}, or axons might be routed correctly at the chiasm but subsequently fail to invade the ipsilateral LGN on arrival. We therefore examined the pattern of labelling of ipsilateral and contralateral optic tracts immediately adjacent to the optic chiasm at times when most afferents have not yet grown into the LGN. Figure 3 gives examples of brightfield autoradiographs from fetuses aged between E30 and E32. Substantial label in normal animals is present within the ipsilateral optic tract by E30 (Fig. 3*a*, arrows), roughly 5 days before the time when ipsilateral afferents unambiguously invade the LGN; label has further increased by E32 (Fig. 3*c*). In contrast, at E31 in Siamese fetuses (Fig. 3*b*), the ipsilateral optic tract is only faintly labelled despite extensive label in the contralateral tract. Thus, in Siamese cats the ipsilateral projec-

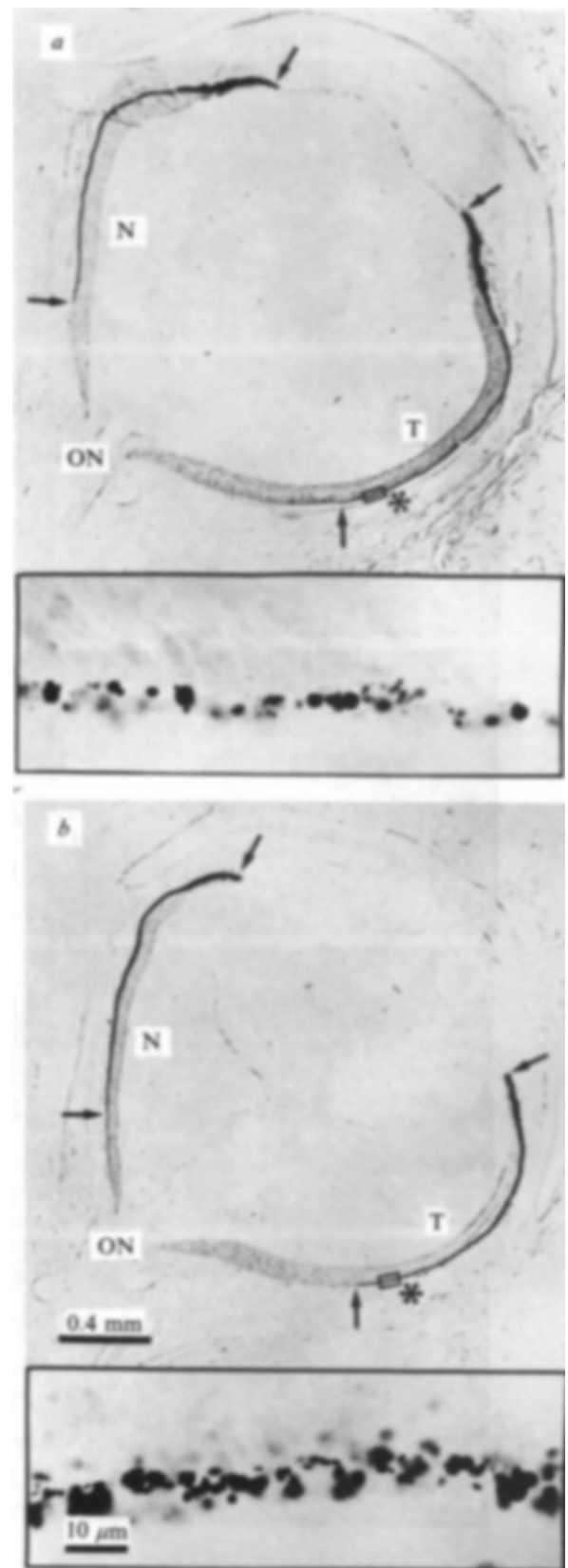


Fig. 4 Brightfield photographs of horizontal sections through the eyes of an E32 Siamese fetus (*a*) and an E32 normal fetus (*b*) to compare the distribution of pigment within the retinal pigment epithelial cells. The sections were counterstained with cresyl violet. Arrows indicate the extent of densely pigmented (black) regions of the epithelium in nasal (N) and temporal (T) retina. ON, optic nerve. Areas enclosed in small rectangles (marked by asterisks) in *a* and *b* are shown at high magnification in the insets beneath to illustrate individual pigment granules that are confined within the pigment epithelial cells. (At these ages, the choroid is unpigmented.)

tion is reduced within the optic tract even before the first retinal afferents reach the vicinity of the developing LGN, thereby supporting the hypothesis that the abnormal projection arises as a consequence of misrouting of growing fibres at the optic chiasm.

It is unknown how the Siamese mutation gives rise to the chiasmatic misrouting of retinal ganglion cell axons seen here. However, it is now well established that an abnormal retinofugal projection is frequently associated with a reduction in adult levels of melanin within the pigment epithelium of the retina. This reduction can be produced by various different mammalian genes, including the Siamese allele¹⁸⁻²⁰. In view of this correlation, it has been suggested that melanin itself or another related gene product of the pigment epithelial cell can influence the chiasmatic fate of retinal ganglion cell axons during fetal life²¹⁻²³. We therefore examined the pattern of pigmentation within the retinal pigment epithelium. Surprisingly, we found that the spatial distribution of pigment in our E32 Siamese cats was similar to that in normal cats of the same age (Fig. 4a, b; compare arrows). However, as can be seen from a comparison of the insets of Fig. 4a, b, the amount of pigment was somewhat reduced in the Siamese fetus. If pigment does have a role within the retina, then these results suggest that it may do so via an actual reduction in pigment levels. Alternatively, a change in the pigmentation of the developing optic stalk, which in other mammals is known to become transiently pigmented during early embryonic development²¹⁻²³, could be the influential factor.

If, as seems likely, optic axons are indeed misdirected at the chiasm in Siamese cats, then it is reasonable to imagine that during normal development, most retinal ganglion cell axons are initially directed to the appropriate side of the brain. It should be emphasized here, however, that our evidence for this conclusion is indirect, as it is based on an examination of the axonal projection pattern. If the present suggestions are substantiated, then the retinogeniculate pathway would represent another example of a system in which directed growth of axons during embryogenesis gives rise to a pattern of connectivity that is retained in the adult. Such directed growth is also found in the development of connections between spinal cord motoneurons and muscles in the chick: motoneurone axons grow directly to the appropriate peripheral muscle masses²⁴. In contrast, in the development of the mammalian neocortex, many cerebral cortical neurones initially elaborate axonal connections with both hemispheres and subsequently retract one but retain the other to become either callosal or association projection neurones in the adult^{25,26}. Thus, it would seem that the nervous system uses very different strategies in the formation of different sets of connections.

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Adult thymectomy does not alter the proportion of T cells of the Ly 123 subclass

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The murine T cell antigens Ly 1, 2 and 3 have been useful in defining functionally distinct T cell subpopulations¹. However, the relationship between Ly 123⁺ cells and Ly 1⁺23⁻ cells is not clear²⁻⁴. It has been claimed^{5,6} that all cells leaving the thymus are Ly 123⁺, the implication being that these must be the precursors of Ly 1⁺23⁻ cells and that maturation involves the loss of Ly 2 post-thymically. Previous work has shown a 50% drop in the proportion of T cells of the Ly 123⁺ subclass following adult thymectomy⁵. The interpretation was that the Ly 123⁺ cells differentiated into Ly 1⁺23⁻ cells and that no Ly 123⁺ migrants from the thymus were replacing them. However, when we directly measured the Ly phenotype of migrant cells within a few hours of their exit from the thymus⁷, we found they were mature in phenotype and already contained Ly 1⁺23⁻ cells in the same proportion (~60%)⁸ as peripheral T cells. This result suggests that adult thymectomy should not affect the subclass distribution of T cells. We have therefore repeated the adult thymectomy experiments using more precise techniques (flow cytometry and monoclonal antibodies) and the results reported here, in contrast to those of the earlier study⁵, did not show any change in the proportion of Ly 123⁺ cells after adult thymectomy, even though total T cell numbers were substantially reduced.

In the original studies⁵ T cells were considered to consist of Ly 123⁺, Ly 1⁺23⁻ and Ly 1⁺23⁺ subclasses, and after adult thymectomy, a 50% drop in Ly 123⁺ cells and an increase in Ly 1⁺23⁺ and Ly 1⁺23⁻ cells was seen. However, it is now clear that all or most T cells express Ly 1, and the major distinction is between Ly 2⁺ (that is, Ly 123 and the so called Ly 23) and Ly 2⁻ (that is, Ly 1) subclasses⁸. Viewed in this light their data showed a 30% drop in Ly 2⁺ cells. For this reason our experiments distinguish T cells primarily on the basis of the presence or absence of Ly 2. With a sensitivity in our assay system of a few per cent, a drop of 30% should be easily detected.

Groups of 4-week-old CBA mice were thymectomized and killed 1, 2 or 4 weeks later. Cells from their mesenteric lymph

Table 1 Proportions of Thy 1⁺ and Ly 2⁺ cells in spleens and lymph nodes of CBA mice at various times after adult thymectomy

		Thy 1 ⁺ as % of viable nucleated cells			Ly 2 ⁺ as % of Thy 1 ⁺ cells		
		8	14	28	8	14	28
Spleen	Control	27	35	36	43	45	39
	TX	20	28	23	40	44	37
Lymph node	Control	70	77	79	38	34	34
	TX	66	70	74	33	34	39

Mice were thymectomized (TX) at 4 weeks of age and assayed 8, 14 or 28 days later. Controls were untreated, aged matched animals. Percentages are based on a sample of 25,000 cells taken from a pool of three animals.

Table 2 Effect of adult thymectomy on T cells in C57BL/6 mice

Organ	Group	No. of animals	% Thy 1 ⁺ (of total cells)	Total Thy 1 ⁺ (% of control)	% Ly 2 ⁺ (of total T cells)
Spleen	Control	4	34	100	44
	Sham TX	4	29	95	43
	TX (a)	3	22	59	44
	TX (b)	3	18	60	47
Lymph node	Control	4	73	100	46
	Sham TX	4	62	92	44
	TX (a)	3	66	38	49
	TX (b)	3	69	49	48

Mice were thymectomized (TX) at 7 weeks of age and assayed 3 weeks later. Controls were untreated age matched animals, while sham TX were age matched animals which were anaesthetized and the chest opened without touching the thymus. Percentages are based on a sample of 50,000 cells taken from a pool of three or four animals. TX (a) and TX (b) are duplicate groups.

nodes or spleens were stained simultaneously with monoclonal anti-Thy 1 and anti-Ly 2. Age-matched normal control mice were similarly stained. The methods and results obtained on a computer-linked fluorescence-activated cell sorter⁹ are shown in Fig. 1. Thy 1⁺ and Ly 2⁺ cells could be unambiguously identified. The staining pattern was highly reproducible, and despite the apparent complexity of the staining protocol, controls were clear cut. The results of this approach are shown in Table 1. At all the times tested after thymectomy, the proportion of Thy 1⁺ cells was reduced (most markedly in spleen as previously noted¹⁰), but the proportion of Ly 2⁺ cells amongst the remaining Thy 1⁺ cells was unchanged.

The original observations of Cantor and Boyse⁵ were made on slightly older (7 week) C57BL/6 mice, so we repeated the experiment with 7-week-old C57BL/6 and CBA mice and, as they did, examined them 3 weeks later. Tables 2 and 3 show the results of these experiments. Three weeks after thymectomy, there was a slight drop in the proportion of T cells amongst the lymphocyte population as a whole and a substantial drop of around 50% in the absolute T cell numbers. Despite these changes the proportion of Ly 2⁺ cells amongst the Thy 1⁺ cells remained constant.

Using a similar staining protocol for Ly 1 and Thy 1, we found there was also no change in the small Thy 1⁻, Ly 1⁺ population 3 weeks after thymectomy (data not shown). This Thy 1⁻, Ly 1⁺ population makes up a few per cent of the cells in normal spleen or lymph node (ref. 8 and R.S., unpublished results). They would fall in the Thy 1⁻, Ig⁺ group in the experiments shown in Fig. 1, but would probably have been included in the Ly 1⁺ group in the experiments of Cantor and Boyse. They do not account for the differences in our results. It is interesting to note that a comparison of the % Thy 1⁺ with the

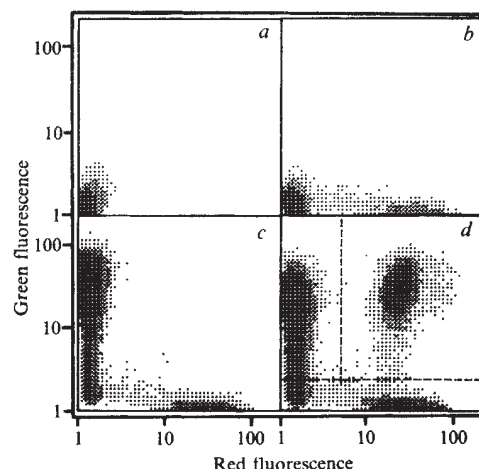


Fig. 1 Typical two-dimensional staining patterns of normal lymph node cells stained simultaneously with anti-Thy 1 and anti-Ly 2 and controls. *a*, Unstained cells; *b*, cells stained with rhodamine-conjugated rabbit anti-mouse immunoglobulin (Rh.RaMlg). B cells can be clearly distinguished from immunoglobulin-negative cells; *c*, cells stained with Rh.RaMlg, followed by rat immunoglobulin to saturate free binding sites on the Rh.RaMlg, followed by fluoresceinated rat monoclonal anti-Thy 1.2 (30-H12, ref. 19). T cells and B cells can be clearly distinguished and a small proportion of double negative cells (null cells) remains; *d*, cells stained with a rat monoclonal anti-Ly 2 (53-6.7, ref. 19) followed by the same staining as *c*. The Rh.RaMlg, which recognizes both mouse immunoglobulin and rat immunoglobulin, now stains both B cells and Ly 2⁺ cells, the latter being distinguished from B cells by the presence of Thy 1. The dotted lines show the levels used to define Thy 1⁺ and Ly 2⁺ cells and the major subpopulations (Thy 1⁺ Ly 2⁺; Thy 1⁺ Ly 2⁻; Thy 1⁻ Ly 2⁺; Thy 1⁻ Ly 2⁻) are easily distinguished. Among the Ly 2⁻ cells, there is no distinct separation between Thy 1⁺ and Thy 1⁻ (*d*). Thy 1⁻ cells were arbitrarily chosen as those with levels undistinguishable from the negative controls (*a* and *b*). The data in each box represent 50,000 cells, plotted as a contour diagram. This form of plotting gives little information on peak heights, but clearly shows degrees of separation. The single peak in *a* contains 50,000 cells, while the other peaks vary (for example, the Ly 2⁺, Thy 1⁺ peak in *d* contains about 17,000 cells). 3×10^6 cells were stained in each case and suspensions were washed between stages. Cells were analysed by flow cytometry (FACS II Becton-Dickenson) using a logarithmically amplified, two colour, single laser system linked to a PDP-11/34 computer. The computer produced the figures shown above and calculated the proportion of cells in each of the categories defined by the dotted lines in *d*. All the numbers in the tables were obtained from this kind of analysis. The slight increase in apparent red staining with increasing Thy 1 levels on red negative cells (*c* and *d*) is an artefact of the electronic signal separation system which occurs when working with low level signals in logarithmic mode.

total Thy 1⁺ (Tables 2, 3) indicates that adult thymectomy must also have caused a substantial drop in non-T (mostly B) cell numbers, indicating that even the adult B cell pool remains to some extent dependent on an intact thymus.

In view of the marked discrepancies with the earlier data two technical questions immediately arise. How do the two techniques for measuring Ly phenotypes differ and was thymectomy in our experiments complete? In the flow cytometric analysis described here monoclonal reagents were used, 50,000 cells were analysed per sample, and background staining levels (that is, rhodamine positive, Thy 1⁺ cells in preparations stained only with anti-Thy 1 and anti-immunoglobulin antibodies, Fig. 1c) were in the range of 1–2%. Thus, the actual numbers of Ly 2⁺ cells counted in various samples ranged from about 17,000 in control lymph node to 5,000 in thymectomized spleens. In the sequential ⁵¹Cr-release cytotoxic analysis, populations of 20–50% were estimated by subtraction following two sequential killing steps each with a background of up to 12%. Furthermore, as different subpopulations may take up different amounts of ⁵¹Cr, the proportion of ⁵¹Cr released may not correspond to the proportion of cells killed. Finally there is the complexity of complement-mediated cytotoxicity, as resistance to lysis can result from factors other than density of surface antigen⁸. For these reasons we believe the flow cytometric analysis to be the more reliable method. With regard to completeness of thymectomy, each animal in our experiments was examined macroscopically and histologically and no evidence of residual thymus was found. Furthermore, the total number of T cells was

Table 3 Effect of adult thymectomy on T cells in CBA mice

Organ	Group	No. of animals	% Thy 1 ⁺ (of total cells)	Total Thy 1 ⁺ (% of control)	% Ly 2 ⁺ (of total T cells)
Spleen	Control	3	29	100	44
	Sham TX	3	31	73	40
	TX (a)	3	25	48	42
	TX (b)	3	23	72	41
Lymph node	Control	3	66	100	36
	Sham TX	3	63	117	35
	TX (a)	3	68	46	37
	TX (b)	3	73	25	35

Mice were thymectomized (TX) at 7 weeks of age and assayed 3 weeks later. Untreated controls and sham TX animals were age matched. Percentages are based on a sample of 50,000 cells taken from a pool of 3 animals. TX (a) and TX (b) are duplicate groups.

reduced by about 50% following the operation, suggesting that thymectomy was complete.

In conclusion, the data reported here are in agreement with the predictions which come from the direct measurement of the Ly distribution^{3,7} and phenotypic maturity^{11,12} of thymus migrants in adult mice. They are also in agreement with other recent reassessments of the Cantor and Boyse model. These showed (1) that there is a significant population of Ly 1 cells in the thymus² and (2) that T cells in newborn mice (which are presumed to be mostly recent thymus emigrants) are similar in Ly phenotype to peripheral T cells in adults^{12,14}. Taken together, these studies support the hypothesis that thymus migrants are mature and that the division between Ly 123⁺ and Ly 1⁺23⁻ cells must occur before emigration from the thymus. In fact, if data showing that Ly 1⁺ cells appear earlier in ontogeny than Ly 123⁺ cells are considered¹⁵⁻¹⁷, it is possible to suggest that the division occurs at the earliest stem cell stage^{2,3,16}.

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Finally, although these data support the hypothesis that thymus migrants in adults are phenotypically mature, it is possible that in some circumstances (for example, at specific stages during ontogeny) the thymus does give rise to migrant cells of 'immature' phenotype, a point which is under investigation^{3,13,18} but not yet resolved. In addition, it should be made clear that these experiments and our other published data concerning thymus migrants^{3,7,11,12} do not necessarily exclude a cortical origin for some or all thymus migrants. Nor do they clarify the relationships of the cortex and medulla. They do however rule out the emigration of immature cells directly from the cortex of adult mice and necessitate a re-analysis of the concept of post-thymic maturation.

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Absence of myosin-like immunoreactivity in stereocilia of cochlear hair cells

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Movements of the stiff sensory hairs, the stereocilia, which extend outwards from the apical surface of the hair cells in the auditory organ, are thought to have a key role in the process of transduction of sound energy into electrical impulses. Each stereocilium is supported by a paracrystalline axial bundle of actin filaments, which have identical polarities and are extensively cross-linked¹⁻⁴. Recent immunohistochemical observations on whole mounts of the guinea pig organ of Corti indicated myosin-like immunoreactivity in association with hair cell stereocilia⁵. Considering the steric situation, it is, however, difficult to imagine any functional interaction between the bundled actin filaments and the assumed myosin molecules. Here we report that in semi-thin, transverse sections of quick-frozen, freeze-dried and plastic-embedded guinea pig organ of Corti, myosin-like immunostaining was restricted to the apical cytoplasm of hair cells and was not detected along the stereocilia. With respect to the immunohistochemical distribution of actin, myosin and the muscular Z-line protein, α -actinin, the apex of hair cells strongly resembles the intestinal epithelial brush border^{6,7}.

Specific immunostaining of cochlear hair cells was achieved using antibodies directed against purified non-muscle myosin from calf thymus (Fig. 1a, b); these antibodies have previously been shown to react with a broad spectrum of non-muscle cells⁸⁻¹⁰. Immunostaining was completely abolished when the antibodies were absorbed with an excess of thymus myosin. The bulk of myosin-specific immunofluorescence was confined to the apex of the hair cells, the so-called cuticular plate (Fig. 2a). A narrow, rather faint, fluorescent band extended from the cuticular plate downwards along the lateral cell border of the outer but not the inner hair cells. Antibodies to striated muscle (chicken pectoral) and smooth muscle (human uterus, chicken gizzard)¹¹ myosin did not react with either the hair cells and their stereocilia or any other cell type of the organ of Corti,

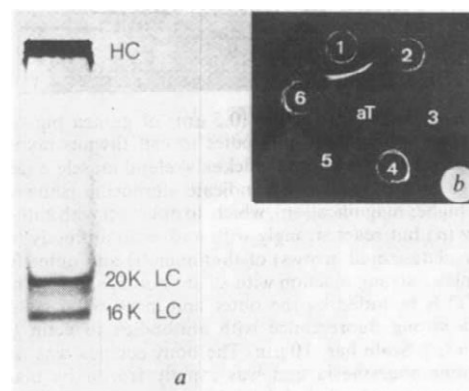


Fig. 1 a, 0.1% SDS-(10%) polyacrylamide gel electrophoresis of calf thymus myosin which was further purified by DEAE-cellulose ion-exchange chromatography and used to prepare antibodies. HC, myosin heavy chain; 20K LC, 20,000-*M_r* myosin light chain; 16K LC, 16,000-*M_r* myosin light chain. b, Double immunodiffusion of anti-calf thymus myosin (aT, centre well) against thymus myosin (1), chicken gizzard (2) and pectoral myosin (3); human uterine (4) and placental (5) myosin, and porcine arterial myosin (6).

indicating that these cells contain an immunologically distinct type of myosin. This finding is in contrast to the observations of Macartney *et al.*⁵, who described staining of cochlear stereocilia with antibody to human uterus myosin. Although we cannot offer a definite explanation for this discrepancy, there are two possibilities to consider. (1) The myosin preparation used by Macartney *et al.*^{5,12} contained minor amounts of some other proteins running in SDS-polyacrylamide gel electrophoresis between the heavy and presumed light chains of myosin (Macartney *et al.*¹² discussed the possibility of contamination of their antigen with actin or tropomyosin) and it is

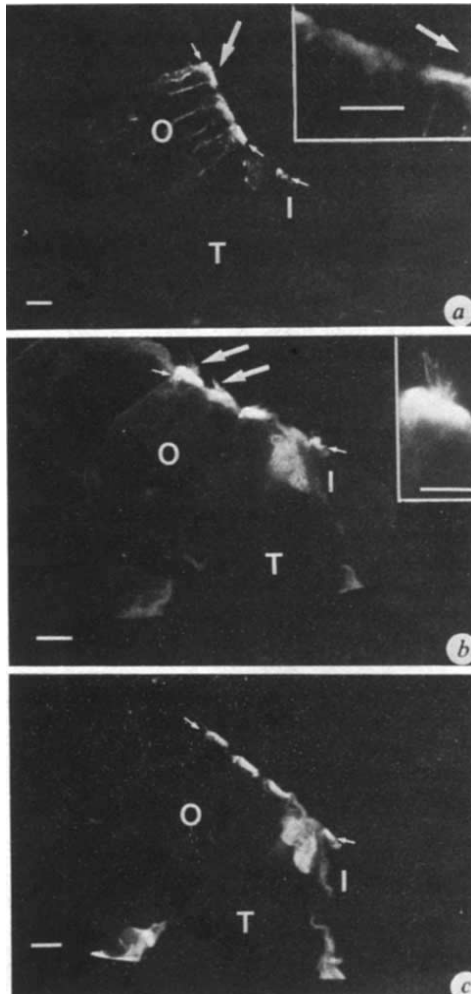


Fig. 2 *a-c*, Semi-thin section (0.5 μm) of guinea pig organ of Corti stained with rabbit antibodies to calf thymus myosin (*a*), chicken gizzard actin (*b*) and chicken skeletal muscle α -actinin¹⁰ (*c*). Large arrows in *a* and *b* indicate stereocilia (shown in the insets at higher magnification), which do not react with anti-myosin antibody (*a*) but react strongly with anti-actin antibody (*b*). The cuticular plate (small arrows) of the inner (I) and outer (O) hair cells display a strong reaction with all antibodies shown. The inner tunnel (T) is bounded by the outer and inner pillar cells which exhibit a strong fluorescence with antibodies to actin (*b*) and α -actinin (*c*). Scale bar, 10 μm . The bony cochlea was removed under ether anaesthesia and was rapidly frozen by placing in melting isopentane cooled with liquid nitrogen. Freeze-drying was performed at 10^{-4} torr starting with a temperature of -100°C , which was increased stepwise to room temperature over 3 days. Freeze-dried bony cochleae were vacuum-embedded in Epon. Semi-thin sections (cut with glass knives on a Reichert Ultracut microtome) were mounted on glass slides and the resin was removed as described elsewhere¹⁸. The antibody concentrations used were 0.5 mg ml^{-1} for the primary antibodies and 0.1 mg ml^{-1} for the FITC (fluorescein isothiocyanate)-labelled goat anti-rabbit IgG (Miles). Dilution of antibodies and rinsing steps were done in phosphate-buffered saline, pH 7.2 at room temperature.

possible that the antibody to uterus myosin contained additional immunoglobulins directed against these unknown minor protein components. (2) Macartney *et al.*⁵ used whole mounts of the organ of Corti for immunohistochemical staining. This method of tissue handling, however, is sensitive to a particular optical artefact known as 'light piping' of stereocilia that might give the impression of specific immunofluorescence (for detailed discussion, see ref. 13). In the present study we applied immunostaining to 0.5- μm -thick sections of quick-frozen, freeze-dried, plastic-embedded tissue. This method avoids diffusion artefacts and allows examination at high resolution. Considerable light piping will probably not occur in these conditions because the thickness of the sections is within the range of the thickness of an individual stereocilium (0.4–0.8 μm)^{2,3}.

As expected, antibodies to actin from chicken gizzard reacted strongly with both the cuticular plate and the stereocilia of the hair cells (Fig. 2*b*). There was a strong actin-like staining in the cytoplasm of the outer and inner pillar cells and the apical processes of phalangeal cells (both structures did not react with anti-myosin antibody). Antibodies to α -actinin from chicken pectoral muscle¹⁰ produced very intense staining in the cuticular plate of outer and inner hair cells but not in their stereocilia (Fig. 2*c*). As with anti-actin, anti- α -actinin antibody displayed a high affinity for the apical processes of phalangeal cells and for the outer and inner pillars, indicating that both actin and α -actinin are major constituents of the prominent cytoskeletal apparatus present in these supporting cells. In the pillars this apparatus consists of bundles of microtubules intermingled with microfilaments (actin?) and electron-dense reticular material (α -actinin?)¹⁴. The apex of phalangeal processes is filled with electron-dense reticular material and microfilaments¹⁴.

Thus, the present findings provide evidence that actin, myosin and α -actinin are concentrated within the apex of cochlear hair cells. While actin is present both the stereocilia and the cuticular plate, myosin and α -actinin are obviously absent from the stereocilia and are restricted to the cuticular plate. A similar distribution of these contractile proteins has been demonstrated in the intestinal brush border^{6,7,15}. Stereocilia have been shown to react like intestinal microvilli with antibodies to fimbrin (an actin cross-linking protein)⁴ but in the present work and in a previously published study⁴, they apparently did not react with antibodies to villin¹⁶ (a 95,000-molecular weight (M_r) protein that bundles and severs actin filaments in a Ca^{2+} -dependent manner¹⁷). This observation indicates differences in the molecular composition of the actin filament bundle present in stereocilia and microvilli. Motile events have so far not been reported in hair cells and their stereocilia. Therefore, it remains to be shown whether myosin and α -actinin serve merely to cross-link and tighten the web of actin filaments within the cuticular plate or whether these proteins constitute a contractile apparatus that might actively modify mechanical properties of the apical portion of cochlear hair cells.

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Meta-vinculin—a vinculin-related protein with solubility properties of a membrane protein

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The localization of vinculin, an actin-binding protein^{1,2} of molecular weight 130,000 (130K) at a variety of cytoskeletal-associated membrane specializations including the zonula adherens of intestinal epithelial cells³, fascia adherens of intercalated discs³, focal adhesion plaques in fibroblasts^{4,5}, and costameres (sarcolemmal domains in periodic register with the I-bands of subjacent myofibrils) in cardiac and skeletal muscle⁶, suggests that vinculin could link actin to cell membranes. This hypothesis is supported by immuno-ultrastructural studies which show that vinculin is closely apposed to the plasma membrane at sites where actin-containing microfilaments terminate^{3,7}. But since vinculin has the solubility properties of a peripheral membrane protein⁴, there would also need to be an integral membrane component to which it bound to link actin filaments to the plasma membrane. Our efforts to identify the presumed membrane binding site for vinculin resulted instead in the detection of a 150K protein which we term meta-vinculin (Greek *meta* among, with; in the sense of closely related to), that is immunologically related to 130K vinculin. Unlike vinculin, meta-vinculin has the solubility properties of an integral membrane protein. Given its location within the cell, it is possible that meta-vinculin is an integral membrane anchor protein for actin filaments.

Unfixed cryostat sections of chicken gizzard were extracted with buffers designed to solubilize either peripheral or integral membrane proteins^{8,9} in an attempt to remove vinculin from its location in dense plaques on the smooth muscle plasma membrane (Fig. 1a)³. The effect of the extractions was assessed by subsequent indirect immunofluorescence staining of the sections with affinity purified anti-vinculin. Because vinculin is isolated by low ionic strength extraction of minced gizzard⁴, for example, with 2 mM Tris/1 mM EGTA, pH 9.0¹⁰ or 1 mM KHCO₃, pH 7.5¹¹, we expected to observe a dramatic decrease in fluorescence when cryostat sections were extracted in these buffers for various times (up to 24 hours) and then fixed and processed for indirect immunofluorescence. However, the low ionic strength buffers extracted barely 20% of the membrane associated vinculin as evidenced both by slightly dimmer staining (Fig. 1c, e) and by microspectrophotometry (data not shown). Neither was vinculin extracted to any greater extent by the following buffers known to disrupt electrostatic interactions between proteins: 50 mM Na acetate pH 5.4 (low pH); 0.01 M NaOH pH 11.5 (high pH); 1.0 M Tris-HCl pH 7.4 (high salt); 0.6 M KI (chaotropic agent); 50 mM Tris plus 0.1–0.6 M KCl pH 8 (buffers of various ionic strengths); 2 mM Tris plus 5 mM EGTA plus 5 mM EDTA, pH 8.0 (chelators). Many integral membrane proteins can be solubilized by non-ionic detergents which primarily disrupt hydrophobic interactions. However, 1% Triton X-100 in 10 mM NaCl, 1.5 mM MgCl₂, 10 mM Tris pH 7.4 (1% Triton buffer) extracted only about 20% of the vinculin from unfixed cryostat sections (Fig. 1g) even though the plasma membranes were disrupted as evidenced by a phase-clear area outlining each smooth muscle cell. Interestingly, the vinculin that remained behind with the detergent-insoluble residue was still organized as in the control sections; the outline of each cell could be clearly discerned by the fluorescence of the anti-vinculin stain. In contrast to all other treatments, we found that over 90% of the vinculin could be extracted by a combination of 1% Triton X-100 and 0.6 M Tris-HCl pH 7.4 (Fig. 1i). These results suggest that to

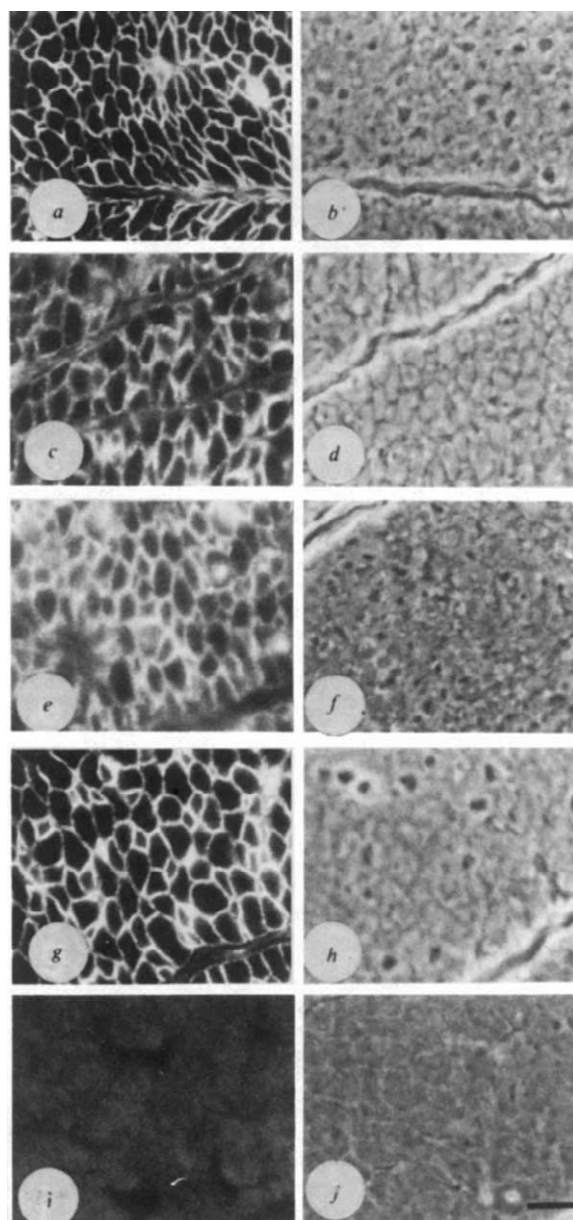


Fig. 1 Conditions for extraction of immunoreactive vinculin from cryostat sections of gizzard. The gizzard from a freshly killed chick was removed, cut quickly into small pieces and dropped into freezing -80°C isopentane. The frozen tissue was transferred to powdered dry ice and frozen onto a copper chuck with OTC compound. $4\text{ }\mu\text{m}$ sections were cut on a Harris cryostat at -20°C and mounted immediately on gelatin-coated glass slides. Sections were extracted in the indicated buffer for the following lengths of time: 1% Triton in 10 mM NaCl, 1.5 mM MgCl₂, 10 mM Tris pH 7.4 or 1% Triton + 0.6 M Tris-HCl pH 7.4, 3 min; 1 mM KHCO₃ pH 7.5 or 2 mM Tris + 1 mM EGTA, pH 9.0, 30 min. After extraction, sections were rinsed in Dulbecco's-PBS (D-PBS), fixed for 5 min in 3% paraformaldehyde pH 7.3 and then processed for indirect immunofluorescence. After incubation with $120\text{ }\mu\text{g ml}^{-1}$ affinity purified anti-vinculin for 45 min, sections were washed extensively in D-PBS. Affinity purified fluorescein conjugated goat anti-rabbit IgG ($50\text{ }\mu\text{g ml}^{-1}$) were then added to the sections. After incubation for 30 min, sections were washed in D-PBS and observed using an Ortholux II fluorescence microscope equipped for epifluorescence illumination with Leitz KG1, BG23, K480 filters and H2 cube. Photomicrographs were recorded with a Vario-Orthomat camera on Kodak Tri-X pan black and white film, ASA 1000. a, b, Unextracted gizzard; c, d, 1 mM KHCO₃ pH 7.5 extraction; e, f, 2 mM Tris/1 mM EGTA pH 9.0 extraction; g, h, 1% Triton pH 7.4 extraction; i, j, 1% Triton + 0.6 M Tris pH 7.4. a, c, e, g, i, Fluorescence micrographs; b, d, f, h, j, phase micrographs. Scale bar, $10\text{ }\mu\text{m}$.

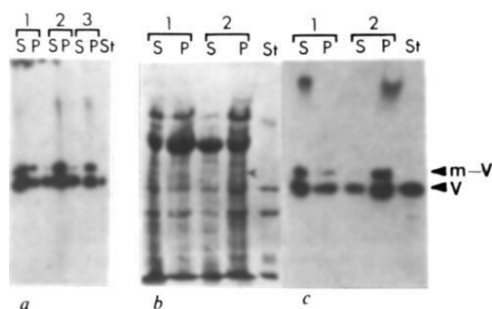


Fig. 2 Detection of 150K meta-vinculin and comparison of its solubility properties with those of 130K vinculin. A 60-mg fragment of gizzard from a freshly killed chick was homogenized in 1.0 ml of the appropriate extraction buffer which included the following protease inhibitors: (final concentration): leupeptin ($10 \mu\text{g ml}^{-1}$), antipain ($20 \mu\text{g ml}^{-1}$), Trasylol ($500 \text{ units ml}^{-1}$), benzamide ($100 \mu\text{g ml}^{-1}$), chymostatin ($10 \mu\text{g ml}^{-1}$) and pepstatin ($10 \mu\text{g ml}^{-1}$). The homogenates were spun at $100,000g$ for 60 min in a SW 50.1 rotor at 4°C . An equal volume of $2\times$ Laemmli SDS sample buffer was added to aliquots of supernatants. $500 \mu\text{l}$ of $2\times$ sample buffer was used to solubilize pellets. Equal volumes of supernatants and pellets were electrophoresed on a 5% Laemmli SDS polyacrylamide mini gel. For immunoblots, the proteins were electrophoresed onto nitrocellulose paper¹². Vinculin and meta-vinculin were detected by incubating the nitrocellulose paper with affinity-purified anti-vinculin ($120 \mu\text{g ml}^{-1}$). After extensive washing in Nonidet P-40 (NP-40) buffer (0.05 M Tris, 0.15 M NaCl, 0.05% NP-40, 0.02% NaN_3 , 0.1% bovine serum albumin pH 8.0), the paper was incubated with ^{125}I -Protein A which was diluted in the NP-40 buffer. The washing was repeated, the paper was dried and then exposed to Kodak XR-5 X-ray film with a Cronex lightening-plus intensifying screen. Immunodetection of proteins in the SDS gel itself was done by the method of Adair *et al.*¹⁸. **a**, Autoradiograph of immunoblot. 1, 1% Triton + 0.6 M Tris-HCl pH 7.4 extraction, supernatant (S) and pellet (P); 2, 1 mM KHCO_3 pH 7.5 extraction; 3, 2 mM Tris/1 mM EGTA pH 9.0 extraction; St, Standards: filamin, 250K; vinculin, 130K; α -actinin, 100K; and bovine serum albumin, 68K. **b**, Coomassie blue stained gel. 1, 1% Triton + 0.6 M Tris-HCl pH 7.4 extraction, supernatant (S) and pellet (P); 2, 1 mM KHCO_3 pH 7.5 extraction. **c**, Autoradiograph of anti-vinculin/ ^{125}I -Protein A overlay of the same gel shown in **b**.

solubilize the bulk of the vinculin it is necessary to disrupt two types of linkages—one electrostatic and the other hydrophobic.

Although these data show that vinculin can be extracted efficiently only in the presence of high salt plus detergent, vinculin is conventionally isolated from a low ionic strength extraction of fresh or frozen chicken gizzard^{4,10,11}. To assess the basis for this apparent discrepancy we extracted fresh gizzards with various buffers in the presence of protease inhibitors and analysed the $100,000g$ supernatants and pellets by SDS gel electrophoresis followed by an immunoblot¹² developed with affinity purified anti-vinculin. The results (Fig. 2) not only confirm the immunofluorescence observations but also reveal that affinity purified anti-vinculin recognizes two molecules: vinculin and a protein (meta-vinculin) with a slightly higher molecular weight than vinculin. The combination of 1% Triton and 0.6 M Tris-HCl pH 7.4 solubilizes most of the vinculin and more than 90% of the meta-vinculin (Fig. 2a-1), whereas the low ionic strength buffers (1 mM KHCO_3 pH 7.5 or 2 mM Tris/1 mM EGTA pH 9.0) extract much less of the vinculin and less than 10% of the meta-vinculin (Fig. 2a-2 and 2a-3). Figure 2b,c shows that the meta-vinculin detected by an autoradiograph co-aligns with a distinct Coomassie blue stained band (Fig. 2b, arrow) which has the appropriate solubility properties of meta-vinculin. In this experiment (Fig. 2c), as well as in several but not all other experiments, we also detected a very high molecular weight ($>250,000$) protein that also reacts with anti-vinculin. Because its presence is variable and unpredictable, the nature of this very high molecular weight cross-reactive material remains unclear. The apparent molecular weight of meta-vinculin was estimated on SDS

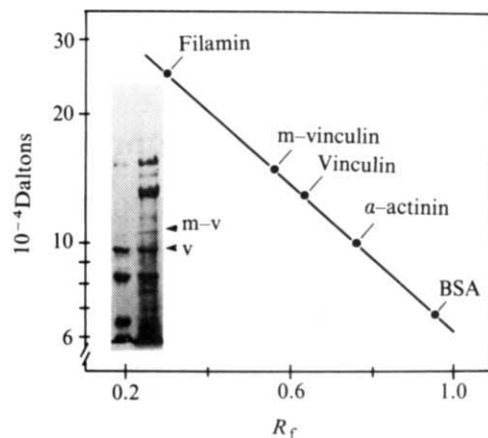


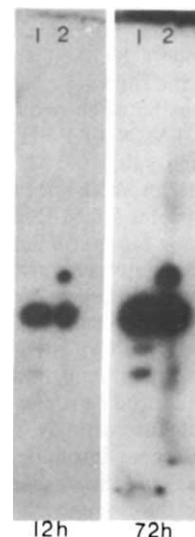
Fig. 3 Estimation of the molecular weight of meta-vinculin by SDS gel electrophoresis. A $100,000g$ supernatant of a 1% Triton + 0.6 M Tris-HCl pH 7.4 extract (prepared as described in Fig. 2) was run on a 5% polyacrylamide mini gel. Meta-vinculin and vinculin were localized in the gel by the antibody overlay technique described by Adair *et al.*¹⁸. Lane 1, standards: Filamin, 250K; vinculin, 130K; α -actinin, 100K; bovine serum albumin, 68K. Lane 2, supernatant of 1% Triton + 0.6 M Tris-HCl pH 7.4 extract. Vinculin and meta-vinculin are marked by arrowheads.

gels under reducing conditions and is approximately 150,000 (Fig. 3).

To rule out the possibility that detection of the 150K protein was due to the presence of contaminating antibodies, we affinity purified vinculin antibody from immune serum in the following manner¹³: vinculin was electrophoresed on a 5% preparative SDS gel which gave good separation of vinculin from meta-vinculin. The 130K band was excised from the gel, and electrophoresed onto nitrocellulose paper, which was then used to affinity purify anti-130K vinculin. An immunoblot of the $100,000g$ supernatant of a 1% Triton plus 0.6 M Tris extract of fresh gizzard proves that anti-130K vinculin also reacts with 150K meta-vinculin (Fig. 4).

The basis for the antigenic relationship between vinculin and meta-vinculin is unknown. It is possible that 130K vinculin isolated by published procedures is a proteolytic fragment of the 150K meta-vinculin. Alternatively, meta-vinculin and vinculin may be products of two different genes which share some degree of homology, or of a single gene whose transcript is spliced differentially to yield molecules with common and unique functional properties as is the case for secreted and membrane-bound IgM¹⁴.

Fig. 4 Anti-vinculin affinity-purified on 130K vinculin also recognizes 150K meta-vinculin. Vinculin (0.5 mg) isolated by low ionic strength extraction of frozen chicken gizzards was electrophoresed on a preparative 5% SDS polyacrylamide gel. After straining the gel for 5 min, the 130K vinculin band was cut out. Vinculin was then electrophoretically transferred to nitrocellulose paper. The paper was incubated with anti-vinculin serum overnight and then washed. Antibody which bound to the 130K protein was eluted with 0.05 M acetic acid, neutralized immediately, and then dialysed overnight against phosphate-buffered saline (PBS) pH 7.4. This affinity-purified antibody was then tested for its ability to identify meta-vinculin and vinculin in an immunoblot of the $100,000g$ supernatant of a 1% Triton + 0.6 M Tris-HCl pH 7.4 extract of fresh chick gizzard prepared as described in Fig. 2. Lane 1, vinculin standard; lane 2, supernatant of a 1% Triton + 0.6 M Tris-HCl pH 7.4 extract. Exposure times were 12 h and 72 h.



Feramisco *et al.*¹⁵ described a 152K protein which is restricted to smooth muscle, and is immunologically related to vinculin, but does not appear to be a proteolytic fragment of vinculin. Possibly this 152K protein is the protein meta-vinculin which we have defined on the basis of solubility properties as well as shared antigenicity with vinculin; however, we do not yet have sufficient information to make a comparison. It will also be of interest to determine if there is a relationship between meta-vinculin and chicken gizzard caldesmon¹⁶, a 150K protein which binds F-actin and is regulated by calcium and calmodulin.

Unlike vinculin, meta-vinculin has the solubility properties of an integral membrane protein. Although it is possible that the low solubility of meta-vinculin in any of the tested solvents other than 1% Triton plus 0.6M Tris simply reflects tight binding of a peripheral protein to an integral membrane pro-

tein, it seems more likely that it reflects differences in the type of bond formed with the plasma membrane. Membrane-associated proteins are divided into peripheral or integral protein classes depending on their solubility properties. Those proteins requiring detergent for solubilization (integral membrane proteins) have been found, with few exceptions, to contain a hydrophobic domain which is buried in the lipid bilayer¹⁷. Based on these considerations, we speculate that meta-vinculin might be an integral membrane protein and as such, a strong contender for the role of directly linking actin to the plasma membrane.

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Ca²⁺-free perfusion of rat heart reveals a (Na⁺ + K⁺)ATPase form highly sensitive to ouabain

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The sensitivities of the cardiac (Na⁺ + K⁺)ATPases to inhibition by ouabain have been shown to correlate with the pharmacologically active digitalis concentrations in the various source species¹. The rat heart enzyme is generally considered to be insensitive to ouabain with half-maximal inhibition at ~6 × 10⁻⁵ M ouabain^{2,3} and a K_D for binding³⁻⁷ of 1-6 × 10⁻⁵ M. However, recent data⁵⁻⁷ showed that rat cardiac sarcolemma preparations can bind ouabain with high affinity (K_D 1-3 × 10⁻⁷ M) with no detectable concomitant enzyme inhibition *in vitro* but producing a sustained positive inotropic effect *in vivo*. In the present study, we measured the (Na⁺ + K⁺)ATPase inhibition by ouabain in sarcolemma-enriched fractions obtained from rat hearts relaxed by perfusion with Ca²⁺-free solutions. In these conditions, two enzyme forms having high and low sensitivities to ouabain were found (half-maximal inhibitions with 1.2 × 10⁻⁸ and 6 × 10⁻⁵ M, respectively). Their relative proportion is close to 1. Confirming previous data²⁻⁷, the highly sensitive form was not detected in preparations from hearts maintained at a physiological calcium level. This heterogeneous population of functional (Na⁺ + K⁺)ATPases suggests that, in rat heart, the positive inotropic effect of low doses of ouabain *in vivo*⁵⁻⁹ would act through the inhibition⁹ of the enzyme activity highly sensitive to ouabain as detected *in vitro*.

Hearts isolated from male Wistar rats (240-300 g) were depleted of calcium or maintained at a normal calcium concentration (2 mM). In the former case, the heart was completely relaxed by perfusion either with an EDTA-containing relaxing buffer¹⁰ (pCa 8) or with a Ca²⁺-free Krebs-Ringer bicarbonate buffer (pCa 4.7)¹¹. To maintain a physiological Ca²⁺ concentration, the perfusion was either omitted or performed with a Ca²⁺-containing buffer. Sarcolemmal vesicles were isolated by differential centrifugations. The (Na⁺ + K⁺)ATPase was measured before and after unmasking the total activity with Na-SDS¹² or by reiterative freezings and thawings. As shown in Table 1, the preparation used in the present work is highly enriched in sarcolemmal vesicles with specific activities of

(Na⁺ + K⁺)ATPase varying between 40 and 100 μmol of phosphate liberated per mg of protein per h. The sensitivity of (Na⁺ + K⁺)ATPase to ouabain was checked with 10⁻⁹ to 2 × 10⁻³ M ouabain (Boehringer) (prepared daily).

Native sarcolemmal vesicles isolated from relaxed hearts exhibited a two-step dose-response curve to ouabain (Fig. 1, curve a): 55 ± 7% of the (Na⁺ + K⁺)ATPase activity was inhibited between 10⁻⁹ and 3 × 10⁻⁷ M ouabain, the half-maximal inhibition (IC₅₀) occurring with 1.2 ± 0.2 × 10⁻⁸ M ouabain. A plateau associated with 45 ± 7% of the total activity was found between 3 × 10⁻⁷ and 10⁻⁵ M, 50% inhibition of this residual activity was obtained with 6 ± 2 × 10⁻⁵ M ouabain. This latter value is consistent with previous data on the insensitivity of rat heart (Na⁺ + K⁺)ATPase to ouabain^{1-9,13} but involves only 45% of the enzyme activity.

A similar biphasic curve having the same proportion of the (Na⁺ + K⁺)ATPase forms was obtained with sarcolemma vesicles subfractionated by centrifugation on sucrose¹⁴ (Fig. 1, curve a). (Specific activity of (Na⁺ + K⁺)ATPase: 205 ± 18 μmol phosphate h⁻¹ mg⁻¹.)

Sarcolemmal vesicles from hearts maintained at a physiological Ca²⁺ level yielded a homogeneous population of (Na⁺ + K⁺)ATPase exhibiting a low sensitivity to ouabain (IC₅₀ 4 ± 2 × 10⁻⁵ M) (Fig. 1, curve b) confirming all previous results^{1-9,13}. Nevertheless, the yields in microsomal proteins and 5'-nucleotidase activity (a sarcolemma-bound marker¹⁵) were lower than those from hearts perfused with Ca²⁺-free solutions, whereas the specific activity of the (Na⁺ + K⁺)ATPase remained constant (Table 1). These results are consistent with the observations of Zak *et al.*¹⁶ who showed that the initial relaxed state of myofibrils facilitated the isolation of subcellular fractions in higher yield and with less destruction. However, as the specific activity of the (Na⁺ + K⁺)ATPase remained constant, one might suppose a conversion of the high affinity form into the low affinity one exhibiting the same molecular turnover. In considering both the specific activity, and the protein yield, the total (Na⁺ + K⁺)ATPase activity in Ca²⁺-containing hearts represented half that found in completely relaxed hearts. In the former case, the enzyme form highly sensitive to ouabain is lost or exists as a catalytically inactive conformation as suggested by Adams *et al.*⁶.

Three more possibilities had to be excluded before concluding that it is the initial low Ca²⁺ level before preparation that reveals a (Na⁺ + K⁺)ATPase activity highly sensitive to ouabain.

First, a variable fraction of the vesicles used in this study may have an inside-out polarity; the curves may reflect differences in accessibility of ouabain to its internalized binding site or

Table 1 Distributions of proteins and enzyme activities in sarcolemmal vesicles

	Ca ²⁺ -free perfused heart			Heart maintained at 2 mM Ca ²⁺		
	Protein yield	(Na ⁺ + K ⁺)ATPase	5' Nucleotidase	Protein yield	(Na ⁺ + K ⁺)ATPase	5' Nucleotidase
Sarcolemmal vesicles						
Native	1.2 ± 0.1%	60 ± 11 (36%)	5.4 ± 1 (33%)	0.6 ± 0.1%	40 ± 10 (12%)	1 ± 0.1 (3%)
Opened	/	100 ± 9 (60%)	16 ± 2 (96%)	/	95 ± 15 (30%)	1.6 ± 0.1 (5%)

Animals were killed by cervical dislocation, the heart was removed and either perfused with Ca²⁺-free medium or maintained at a normal Ca²⁺ level (2 mM). Ca²⁺-free hearts were perfused via coronary arteries for 1 min at room temperature with 5 ml of the ice-cold relaxing buffers^{10,11}. Hearts at normal Ca²⁺ levels were either perfused in the same conditions with an ice-cold solution containing 2 mM CaCl₂, 25 mM KCl and 39 mM sodium tetraborate/HCl pH 6.8, or kept in ice for 5 min. The minced ventricles were homogenized at 4 °C in 30 ml of 20 mM sodium pyrophosphate (PP_i), 0.1 mM phenylmethanesulphonylfluoride, 10 mM Tris-HCl pH 7.8, with a Polytron PT 20 (2 × 0.5 s, setting 5). After stirring for 20 min at 4 °C, the suspension was made up to 120 mM KCl, 30 mM NaCl and 250 mM sucrose by adding concentrated solutions. The 500g 5-min supernatant was diluted to 120 ml of this isotonic and isosmotic solution, adjusted to pH 6.8 with 40 mM imidazole-HCl, and centrifuged at 7,500g for 15 min and then at 31,000g for 30 min. The final pellet was washed in 50 mM PP_i, 135 mM NaCl, 5 mM MgCl₂ and 5 mM Tris-HCl pH 7.4 to eliminate myofibrils. The sarcolemmal vesicles were resuspended in 100 mM NaCl, 250 mM sucrose and 40 mM imidazole-HCl pH 6.8 and analysed for enzyme content. The (Na⁺ + K⁺)ATPase activity was assayed at 37 °C in a medium containing 10 µg sarcolemma protein²⁷, 4 mM MgCl₂, 100 mM NaCl, 10 mM KCl, 40 mM imidazole-HCl pH 7.4. The reaction was initiated with the addition of prewarmed ATP (final concentration 4 mM) and carried out over six time points to assess linearity. Aliquots were taken every 30 s and the released orthophosphate was measured²⁸. The (Na⁺ + K⁺)ATPase activity was taken as that inhibited by 2 × 10⁻⁵ M ouabain. The activities of the (Na⁺ + K⁺)ATPase reported here represented 30–50% of total ATPase activities. 5'-Nucleotidase activity¹⁵ was assayed for 1 h at 37 °C in a medium containing 20–100 µg protein ml⁻¹, 100 mM KCl, 10 mM MgCl₂, 5 mM AMP and 50 mM imidazole-HCl pH 7.4. This activity was taken as that insensitive to 10 mM potassium sodium tartrate²⁹ but that inhibited by 1 mM adenosine 5'-diphosphonate. Activities are expressed as µmol orthophosphate per mg of protein per h. Opened vesicles were obtained by pretreatment with Na-SDS¹² or by reiterative freezings and thawings: samples of freshly prepared sarcolemma vesicles (~0.1 mg) were frozen at -40 °C for 24 h, thawed, analysed for their enzyme content and frozen again. This procedure was performed twice. Values in parentheses give recovery of enzyme activity as % of total patent activity of homogenate. Whatever the pretreatment of the heart, the specific activities of (Na⁺ + K⁺)ATPase and 5'-nucleotidase in the ventricle homogenates were 2.1 ± 0.5 and 0.2 ± 0.06 µmol phosphate per h per mg, respectively. Note that in the intermediate fractions obtained along with the purification procedures, no significant (Na⁺ + K⁺)ATPase and 5'-nucleotidase activities could be measured.

variations in the proportion of inverted vesicles in the sarcolemma preparations. This possibility was excluded as a pretreatment of the vesicles, whatever their source, with Na-SDS or by reiterative freezings had no effect on the dose-response curves.

Second, the sensitivities of the (Na⁺ + K⁺)ATPase activity to ouabain seemed not to be due to drastic differences in the rates of drug binding to both native and opened vesicles. In the absence of ATP, either a 10-min or a 30-min equilibration period at 37 °C with varying doses of the drug led to the same inhibitions of the enzyme activity. In the presence of ATP, which favours ouabain binding¹⁷, the amounts of phosphate

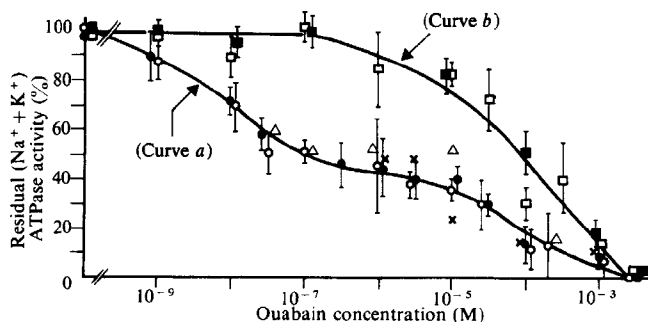


Fig. 1 Dose-response curves of (Na⁺ + K⁺)ATPase activity versus ouabain concentration. Curve a, Ca²⁺-free perfused heart: native (●) and opened (○) sarcolemmal vesicles from ventricle, opened vesicles subfractionated on sucrose¹⁴ (×), and opened vesicles (△) from isolated myocytes^{11,28}. Curve b, heart maintained at 2 mM Ca²⁺: native (■) and opened (□) sarcolemmal vesicles. The sensitivity of (Na⁺ + K⁺)ATPase was checked as follows. After incubation of sarcolemmal vesicles for 30 min at 37 °C with various amounts of ouabain in the ATP-free assay medium, ATP was added to initiate the enzymatic reaction. The activity at a given ouabain concentration (x) is: [(activity)^x - (activity)^{2 × 10⁻⁵ M}] / [(activity)⁰ - (activity)^{2 × 10⁻⁵ M}] × 100. Whatever the pretreatment to which the heart was subjected, neither of our data showed net stimulation of the enzyme at any concentration. Each point represents the mean of 4–18 observations with bars denoting s.e.m. computed from percentage inhibition in each experiment. Each experiment was performed at least four times with at least four different sarcolemmal preparations.

liberated over the assays were linear with respect to time, suggesting that the reversible enzyme-ouabain equilibrium^{2,3} was reached in less than 30 s (the shortest time used) even in the presence of 10 mM KCl.

Third, the enzyme form highly sensitive to ouabain could be associated with cell membranes from more than one cell type¹⁸. The experiments with sarcolemma vesicles from isolated myocytes¹⁹ from relaxed hearts¹¹ ruled out this alternative explanation. The two-step inhibition by ouabain was the same as in sarcolemma preparations from the whole ventricle (Fig. 1, curve a). In isolated myocytes, Adams *et al.*⁶ reported a high affinity binding site for ³H-ouabain with a *K_D* of 1.2 × 10⁻⁷ M. Our process of isolation may have changed the properties of the enzyme in relation to the effect of the glycoside, that is, an increased sensitivity. However, the disparity with our data may be due to calcium loading as a result of the long-lasting incubation of the myocytes at 37 °C with 0.5 mM Ca²⁺. This would lead to a situation different from the physiological conditions (intact heart, 2 mM external calcium and 10⁻⁵–10⁻⁷ M internal free Ca²⁺) and the conditions used here (sarcolemma vesicles, 3 × 10⁻⁶ M free Ca²⁺). Thus, the dose-response curve for the low ouabain concentration reported by Adams *et al.*⁶ would represent the sum of both high and low sensitivity enzyme forms in an unknown proportion depending on the internal Ca²⁺ pools and/or the size of the rapidly exchanging calcium stores²⁰.

These parameters would control the expression of the high sensitivity enzyme form. Such an effect of Ca²⁺ on the effects of ouabain is consistent with the different relationships between ouabain and the systolic (10⁻⁵ M internal free Ca²⁺) and diastolic (10⁻⁷ M internal free Ca²⁺) tensions in rat heart⁷. Further, the experiments of Carrier *et al.*²¹ show that in guinea pig heart preparations, the relative inotropic action of ouabain is much larger when the Ca²⁺ store is small than when it is large. A direct effect of millimolar external Ca²⁺ levels on the expression of the two enzyme forms cannot be excluded but is very unlikely since our observations are consistent with the biphasic inotropic action of ouabain (10⁻⁸–10⁻⁶ M and 10⁻⁴ M) in isolated rat hearts perfused with 1.4 mM Ca²⁺ (ref. 22).

There is some evidence that two components could be involved in the inotropic effect of ouabain *in vivo*^{5–8,22} as if the high- and low-affinity ouabain-binding sites detected in rat heart sarcolemmal vesicles were two (Na⁺ + K⁺)ATPase isozymes⁶

being inhibited by the drug in a concentration range between 3×10^{-8} M and 10^{-3} M'. Alternatively, a single class of enzyme molecules may express one or two sensitivities to ouabain depending both on the Ca^{2+} concentrations^{23,24} and on the interactions with other membrane proteins^{24,25}.

The present results provide further insight into the therapeutic mechanisms of ouabain²⁶; the high sensitivity enzyme form might represent the physiological site responsible

for the positive inotropic effect of low concentrations of ouabain *in vivo*.

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Minute virus of mice inhibits cell transformation by simian virus 40

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Certain paroviruses have been reported to inhibit spontaneous¹ or virus-induced² tumorigenesis in rodents; the mechanisms of this antineoplastic activity is unknown. We have investigated the ability of the minute virus of mice (MVM), a non-defective parovirus³, to interfere with the *in vitro* transformation of mouse cells by simian virus 40 (SV40). We used variants of BALB/c 3T3 cells, denoted PVR, selected for their resistance to the lytic effect of MVM. We report here that inoculation of SV40-infected PVR cells with MVM reduced dramatically the yield of transformed clones. Moreover, stable SV40 transformants of PVR cells lost up to 85% of their ability to grow in soft agar after MVM infection. Our results indicate that the antineoplastic effect of paroviruses can be simulated in cell cultures and may involve a direct and selective toxic effect of these viruses on malignant cells or their precursors.

In mature animals, paroviruses interfere with the development of tumours but are, in general, innocuous for normal cells regardless of their mitotic activity³. It is unclear whether this selective inhibition of oncogenesis depends on complex events occurring in whole animals such as immunological, hormonal or interferon induction responses. We therefore investigated whether the antineoplastic activity of paroviruses could be reproduced and analysed *in vitro* in the absence of these physiological processes. An inhibition of the oncogenesis of adenovirus⁴- or herpes simplex virus⁵-transformed cells was achieved by exposing *in vitro* cultures of these cells to adeno-associated virus (AAV), a defective parovirus, before inoculation into animals. Adenovirus and herpes simplex virus are the only two viruses that can serve as helper for the replication of AAV. Thus, it is questionable whether the antineoplastic activity of AAV results from its relationship to the helper virus or from a direct interaction between the parovirus and the transformed cell *per se*. We tested the latter possibility by investigating whether the non-defective parovirus MVM interfered with *in vitro* cell transformation by SV40, a virus without discernible helper activity for paroviruses.

The BALB/c 3T3 mouse cell line⁶ was used as it is highly susceptible to SV40 transformation. These cells are normally sensitive to MVM lytic infection, as are other established cultures of mouse fibroblasts. To mimic the *in vivo* situation we isolated from BALB/c 3T3 cells a series of variant clones resistant to MVM infection. These clones were denoted PVR (parvovirus-resistant). Typical properties of PVR cells are illustrated in Table 1. The PVR cells resisted viral attack as shown by both high cell survival and low production of infectious progeny virus. In contrast, PVR cells did not differ significantly from the permissive parent cells with respect to MVM uptake (data not shown), suggesting that their resistance occurred at an intracellular step of MVM replication.

Table 2 (columns *a* and *b*) shows that the PVR strains tested were susceptible to transformation by the oncogenic virus SV40 as measured by the ability of SV40-infected cells to form colonies in soft agar. The transformation frequency of PVR cells inoculated with SV40 (100 plaque-forming units (PFU) per cell) ranged from 1 to 5% and was equivalent to that of parental BALB/c 3T3 cells. The selection of PVR cells therefore had no detectable effect on their susceptibility to *in vitro*

Table 1 Susceptibility of BALB/c 3T3 and PVR-1 cells to MVM infection

Multiplicity of infection (PFU per cell)	Surviving fraction relative to uninfected cells		Fraction of inoculated cells producing infectious centres	
	BALB/c 3T3	PVR-1	BALB/c 3T3	PVR-1
1	<10 ⁻³	0.99	0.41	0.008
10	<10 ⁻³	0.90	—	—
100	<10 ⁻³	0.78	0.66	0.05

The PVR-1 strain was cloned from a population of BALB/c 3T3 cells⁶ surviving two successive infections with 100 PFU of MVM per cell. Cells from the parental BALB/c 3T3 line and from the PVR-1 strain were inoculated with increasing amounts of MVM. After 1 h, the inoculum was removed and extracellular virus was inactivated by incubation for 3 h in medium containing rabbit anti-MVM serum⁷ (given by P. Tattersall). Cultures were then collected and processed for cell survival and infectious centre assays. Cell survival was measured by colony formation on plates and is expressed relative to uninfected cells. The plating efficiencies of uninfected BALB/c 3T3 and PVR-1 cells were 0.35 and 0.27, respectively. The fraction of productively infected cells was measured by plaque formation after seeding of MVM-inoculated cells on to appropriate indicator cells⁷. The resistance of PVR cells to MVM attack was maintained during continuous subculturing for at least 1 yr.

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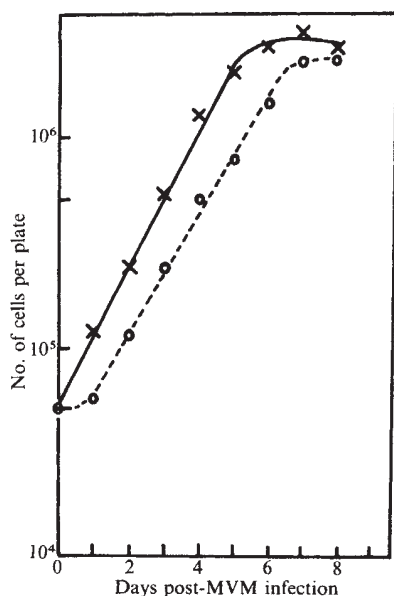


Fig. 1 Effect of MVM on the growth of monolayer cultures of SV40-transformed PVR-1 cells. SV40-transformed PVR-1 cells in 60-mm dishes were inoculated with MVM (100 PFU per cell; (○) or mock-infected (×). Cultures were further incubated in liquid medium containing rabbit anti-MVM serum⁷ and collected at various intervals. Cells were counted with a haemocytometer.

transformation. Clearly, MVM reduced dramatically the yield of *in vitro* transformation of PVR cells previously infected by SV40 (Table 2, columns *c* and *d*). In order to identify which step of the transformation process is the target for MVM, we isolated stable SV40 transformants of PVR-1 cells and tested their sensitivity to MVM. Table 3 shows that MVM infection decreased markedly the viability of SV40-transformed PVR-1 cells as measured by their cloning efficiency on plastic plates. As SV40 transformation did not increase the plating efficiency of PVR-1 cells (see Tables 1 and 3), this effect can be accounted for, not by the reversion of the transformed phenotype, but rather by a cytotoxic action of MVM on transformed cells. Although 75% of the transformed PVR-1 cells were killed after inoculation with MVM at a multiplicity of 100 PFU per cell, only 16% of these cells supported a productive MVM infection (Table 3). However, the yield of infectious centres was 3–6 times greater for SV40-transformed (Table 3) than for normal (Table 1) cells. Thus, SV40 transformation was apparently associated with a greater sensitivity to the cytotoxic effect of MVM although only a very small fraction of transformed cells was fully permissive to this virus. The abortion of the productive infection in a fraction of the sensitive cells is a common, yet quantitatively variable, feature of parvovirus replication, as illustrated in Table 1 for BALB/c 3T3 cells. Table 3 also indicates that the cloning efficiency of stable SV40 transformants of PVR-1 cells in soft agar was reduced by 85% after infection with MVM at a multiplicity of 100 PFU per cell. As SV40-inoculated PVR-1 cells were prevented from forming clones in soft agar to a similar extent by MVM co-infection (Table 2), the data suggest that the presence of MVM during the establishment of the transformed phenotype is not essential for the inhibition of *in vitro* cell transformation.

The effect of MVM on the growth of monolayer cultures of SV40-transformed PVR-1 cells was studied in conditions that prevented secondary MVM infections. Figure 1 shows that MVM caused a delay in the proliferation of transformed PVR-1 cells. This lag was not detectable for non-transformed cells (data not shown) and probably reflects the killing of a fraction of the cells as a result of MVM infection (Table 3). SV40-transformed PVR-1 cells that survived MVM inoculation were not intrinsically resistant to MVM as they could be superinfected and showed the same growth delay as the parental culture (data not shown). Cell survivors contributing to the proliferation

Table 2 Effect of MVM on *in vitro* transformation of PVR cells by SV40

Cells	Cloning efficiency in soft agar(%)			<i>d</i> , MVM inhibition of SV40 transformation (%)
	<i>a</i> , No virus	<i>b</i> , +SV40	<i>c</i> , +SV40 +MVM	
PVR-1	$<5 \times 10^{-5}$	3.6	0.38	89.5
PVR-2	$<5 \times 10^{-5}$	2.4	0.37	84.2
PVR-3	$<5 \times 10^{-5}$	1.7	0.05	97.1

PVR-1, -2 and -3 strains were cloned from a population of BALB/c 3T3 cells surviving MVM infection and achieved similar levels of resistance to MVM (see Table 1). PVR strains were tested for their anchorage-independent growth ability, as measured by the formation of foci in soft agar⁹. *a*, Uninfected cells; *b*, cells inoculated with SV40 (100 PFU per cell) and propagated for six generations before suspension in agar-containing medium; *c*, same as *b*, except that cells were superinfected with MVM (100 PFU per cell) 24 h after SV40 inoculation. *a* and *b* series underwent mock infections. Intervals between SV40 and MVM infections ranging from 0 to 24 h were tested and gave essentially the same results (data not shown). Foci were counted 4 weeks after suspension in soft agar and were of similar size in all series. Incubation of MVM-treated cells for longer periods did not increase cloning efficiency.

of the culture after the 1-day lag period did not differ markedly from uninfected cells with respect to their saturation density and growth rate (Fig. 1). It can be calculated from Fig. 1 that the generation time of MVM-infected cells was ~24 h, less than 1.5 h longer than for uninfected cells. Thus, the major restriction imposed by MVM on SV40-transformed cells seems to occur at the level of cell viability rather than growth properties. Recently, opposite conclusions were reached for the action of the defective parvovirus AAV on adenovirus-transformed cells. This discrepancy could be due to the fact that adenovirus is a helper for AAV replication. This interrelationship imposes specific restrictions on the expression of both viruses after AAV infection of adenovirus-transformed cells⁴ and might account for the selective interference of AAV with the oncogenicity of its helper viruses^{4,5}. Thus, the mechanisms underlying the inhibition of *in vitro* transformation might be at least partly different for defective AAV^{4,8} and for autonomous MVM parvoviruses.

The results presented here indicate that one pathway of parvoviral surveillance against cancer might involve a direct and selective inactivation of transformed cells by these viruses. The privileged interaction of parvoviruses with transformed cells strongly suggests that transformation sensitizes cells that are normally resistant to virus infection, although a preferential transformation of pre-existing parvovirus-sensitive cells cannot be ruled out. It has been shown recently that the cellular permissivity to lytic parvovirus replication is controlled by developmentally regulated host factors⁷. The relevance of our

Table 3 Effects of MVM on SV40-transformed PVR-1 cells

Multiplicity of infection (PFU per cell)	Cell surviving fraction	Fraction of productively infected cells	Colony formation in soft agar	
			Cloning efficiency (%)	% Inhibition
0	1	0	7.8	0
0.1	—	—	5	36
1	0.52	0.05	4.1	48
10	0.32	—	2.1	74
100	0.25	0.16	1.2	85

A strain of transformed cells was derived from a clone of SV40-infected PVR-1 cells growing in soft agar (Table 2, column *b*). These cells expressed SV40 T antigen as measured by immunostaining (data not shown). SV40-transformed PVR-1 cells were inoculated with increasing amounts of MVM. Infected cells were then processed for cell survival and infectious centre assays (see Table 1 legend) or incubated for 5 h and suspended in soft agar for transformation assay (see Table 2). Viability and virus production were measured in the same experiment as for normal cells (Table 1). The plating efficiency of SV40-transformed PVR-1 cells was 0.12. The time of appearance and the size of foci in soft agar were not affected by MVM infection.

experimental system to the situation *in vivo* rests on the assumption that the expression of such a factor(s) is limiting in PVR cells as it is unknown why these selected cells fail to replicate MVM. Yet our data raise the possibility that changes in cellular gene expression triggered by neoplastic transformation might lift barriers to parvovirus replication, thereby making malignant cells a preferential target for the cytotoxic action of these viruses. Use of our *in vitro* system for the biochemical analysis of the MVM life cycle should elucidate these barriers. Such a system provides a means of studying the molecular mechanism of the parvoviral antineoplastic activity.

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Oncogenes in solid human tumours

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The identification and isolation of oncogenes capable of inducing malignant transformation on transfection into rodent cells from human tumour cells has opened the possibility of deciphering the processes involved in human carcinogenesis. With the exception of three human lymphomas¹, the human transforming genes so far identified have been detected in established tumour cell lines^{1–8}, raising the possibility that they might have been activated during *in vitro* manipulation of the cells. However, we report here that unmanipulated human solid tumours, including carcinomas of the colon (two), lung and pancreas and an embryonal rhabdomyosarcoma, also contain dominant transforming genes. The carcinomas of the lung and pancreas and the rhabdomyosarcoma possessed a common oncogene which, like that isolated from human LX-1 lung carcinoma cells⁹, shares sequences with the *onc* gene of the Kirsten strain of murine sarcoma virus (MSV). We also show that several other human tumour cell lines, including those established from carcinomas of the colon (A2233), lung (A427 and A2182), gall bladder (A1604) and urinary bladder (A1698), possess the same oncogene. Thus a variety of human tumours, regardless of their clinical manifestations, contain a common transforming gene.

We have isolated high-molecular weight DNA from a variety of surgically removed solid human tumours, some of which have been stored at -70°C for up to 5 yr. These human DNAs were used to transfect NIH 3T3 mouse cells by the calcium phosphate precipitation technique^{10,11}. Foci of morphologically transformed NIH 3T3 cells were picked by the cloning cylinder technique and cloned as single cells in semi-solid media. The clonal cells were grown to mass culture and their DNAs analysed for the presence of human repetitive sequences² (Table 1). About 15% of the human solid tumours tested (5 out of 28) which included carcinomas of the colon (two), lung and pancreas and an embryonal rhabdomyosarcoma from a 1-yr-old

patient, were found to contain dominant oncogenes capable of transforming NIH 3T3 cells. Transformation efficiencies (0.05–0.1 focus per μg of donor DNA per 2×10^5 NIH 3T3 cells) were about fivefold lower than those observed with T24 bladder carcinoma DNA, but comparable with those obtained with DNAs isolated from other human tumour cell lines⁸. Interestingly, DNA isolated from normal tissue of the patient, whose pancreatic carcinoma exhibited an oncogene, failed to induce transformation of NIH 3T3 cells in the same experimental conditions.

After three cycles of transfection, human DNA sequences encompassing the corresponding oncogenes present in each of the above solid human tumours were identified by Southern blot analysis using a probe specific for human repetitive sequences. Representative third-cycle transformants derived from a colon carcinoma of a 66-yr-old female, exhibited two common *EcoRI*-cleaved DNA fragments of about 10 and 7 kilobase pairs (kbp) (Fig. 1A). NIH 3T3 transformants derived from a second colon carcinoma obtained from the ascending portion of the colon of a 57-yr-old male patient also possessed two common *EcoRI*-cleaved human DNA fragments, although of different sizes (25 and 4 kbp; Fig. 1B). Southern blot analysis of these third-cycle transformants with other restriction endonucleases such as *HindIII* and *BamHI* revealed different patterns of human DNA fragments (data not shown), suggesting that these two colon carcinomas contain different transforming genes.

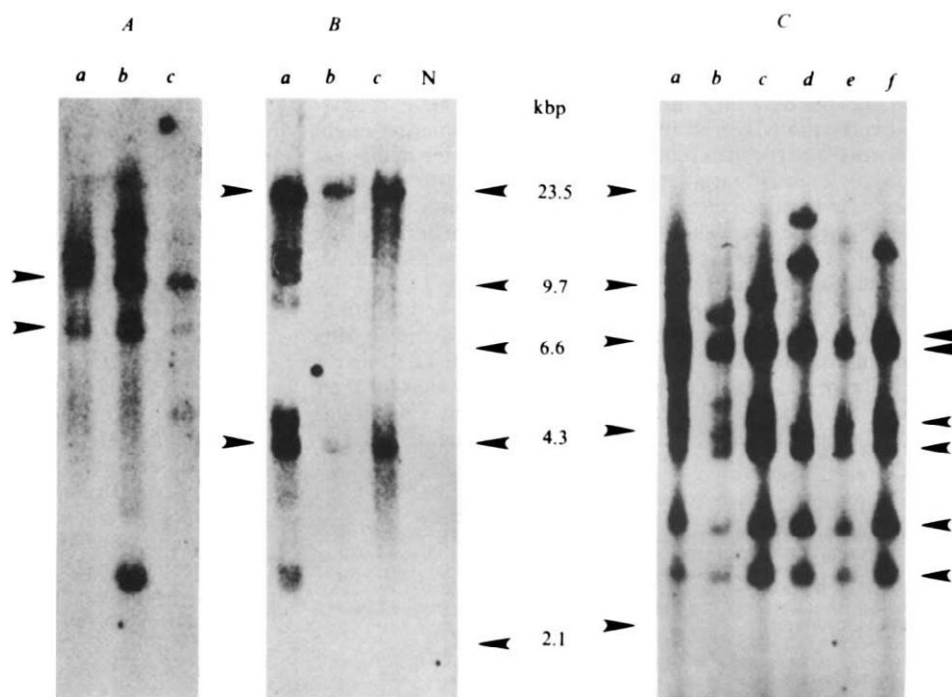
When similar studies were performed with representative third-cycle transformants derived from the lung and pancreas carcinomas and from the rhabdomyosarcoma, a different picture emerged. All the transformants tested exhibited a common pattern of *EcoRI*-cleaved human DNA fragments of about 6.8, 6.7, 4.4, 4.0, 3.0 and 2.4 kbp (Fig. 1C). In analogous experiments, these transformants were found to possess three common *HindIII*-cleaved human DNA fragments of about 18.0, 12.2 and 3.4 kbp (data not shown). These results indicate that three different solid human tumours possess the same, or a highly related, oncogene.

We have also detected the presence of transforming genes in various human tumour cell lines including those established from carcinomas of the colon (A2233), lung (A427 and A2182), gall bladder (A1604) and urinary bladder (A1698 and T24) as well as from a fibrosarcoma (HT-1080). When the human DNA fragments carrying the corresponding oncogenes were characterized by submitting the DNAs of representative third-cycle NIH 3T3 transformants to Southern blot analysis, we observed that colon carcinoma A2233, lung carcinomas A427 and A2181, gall bladder carcinoma A1604 and urinary bladder carcinoma A1698 cells also contained the same pattern of human DNA fragments found in the oncogene present in the three solid human tumours described above (data not shown). Thus of the 12 human tumours (5 solid tumours and 7 established tumour cell lines) in which we have identified dominant transforming genes, 8 (67%) possess the same, or a highly related, oncogene.

Recently, Perucho *et al.* have reported that three human tumour cell lines established from lung and colon carcinomas contained the same transforming gene⁶. Moreover, this gene was indistinguishable from that previously identified by Murray *et al.* in SW480 colon carcinoma cells⁵. Close examination of the pattern of *EcoRI*-cleaved fragments of this lung/colon carcinoma oncogene and that depicted in Fig. 1C for the oncogene identified here in eight different tumours strongly suggests that they are the same oncogene.

It has been recently demonstrated that a human transforming gene isolated from T24 and EJ bladder carcinoma cells^{8,12,13} is an activated form of the normal human homologue of the *onc* genes of the BALB and Harvey strains of murine sarcoma viruses (MSV)^{9,14,15}. Furthermore, Der *et al.* have shown that the oncogene present in LX-1 human lung carcinoma cells contains a 3.0-kbp *EcoRI* fragment highly related to *kis*, the *onc* gene of the Kirsten strain of MSV⁹. Thus, we decided to

Fig. 1 Southern blot analysis of human repetitive sequences in third-cycle NIH 3T3 transformants derived from solid human tumours. 20 μ g of high-molecular weight DNA isolated from: A, a-c, representative third-cycle transformants obtained by transfection with DNA isolated from colon carcinoma 1665; B, a-c, representative third-cycle transformants obtained by transfection with DNA isolated from colon carcinoma 2033; N, control NIH 3T3 mouse cells; C, representative third-cycle transformants obtained by transfection with DNA isolated from rhabdomyosarcoma 1085 (a, b), lung carcinoma 1615 (c, d) and pancreatic carcinoma 1189 (e, f) were digested with 20 U of *Eco*RI for 1 h at 37°C, electrophoresed (18 h at 30 V) in horizontal agarose (0.7% w/v) gels and blotted to nitrocellulose filters as described by Southern²⁴. Filters were hybridized in stringent conditions (50% formamide, 5 $\times$ SSC and 42°C) for 44 h to 4 $\times$ 10⁷ c.p.m. of nick-translated high-molecular weight human DNA. Hybridized blots were exposed to Kodak XR-5 film at -70°C in the presence of intensifier screens for 2 days. Co-electrophoresed DNA fragments of *Hind*III-digested λ c1857 DNA served as size standards (labelled in kbp). Migration of *Eco*RI-cleaved human DNA fragments shared by third-cycle transformants derived from either A, colon carcinoma 1665; B, colon carcinoma 2033; or C, rhabdomyosarcoma 1085, lung carcinoma 1615 and pancreatic carcinoma 1189 are indicated by arrows.

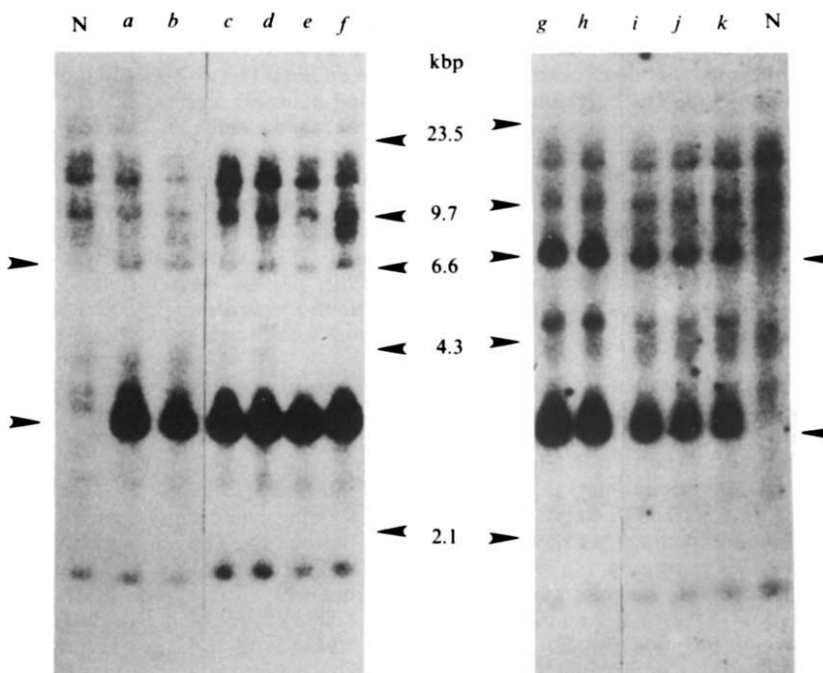


investigate whether the oncogene present in the rhabdomyosarcoma, lung and pancreatic carcinomas, and in A2233 colon, A427 and A2182 lung, A1064 gall bladder and A1698 urinary bladder carcinoma cell lines also contained sequences related to retroviral transforming genes. DNAs isolated from representative third-cycle transformants derived from each of the above tumours were digested with *Eco*RI and submitted to Southern blot analysis for the presence of retroviral *onc* gene sequences. Radioactive probes included those specific for *v-abl* (Abelson murine leukaemia virus), *v-bas* (BALB-MSV), *v-fes*ST (Snyder-Theilen strain of feline sarcoma virus), *v-mos* (Moloney-MSV), *v-myb* (avian myeloblastosis virus), *v-myc* (avian MC29 virus), *v-kis* (Kirsten-MSV), *v-sis* (simian sarcoma virus) and *v-src* (Rous avian sarcoma virus)¹⁵.

The *kis* probe specifically detected the presence of two *Eco*RI-cleaved DNA fragments of 6.8 and 3.0 kbp in the genomes of each of the NIH 3T3 transformants tested (Fig. 2). Neither of these DNA fragments was found in control NIH 3T3 cells, indicating that they were derived from *c-kis*, the human homologue of the *onc* gene of Kirsten-MSV¹⁶. None of the other retroviral probes used detected human DNA sequences present in these NIH 3T3 transformants, with the exception of *v-bas* which hybridized weakly to the 3.0-kbp *Eco*RI DNA fragment of human *c-kis*^{16,17}.

To determine whether the retrovirus-related sequences of this human oncogene were expressed, we analysed NIH 3T3 transformants for the presence of proteins antigenically related to p21, the gene product of Kirsten-MSV¹⁸. Radioactively

Fig. 2 Detection of sequences related to the *onc* gene of Kirsten-MSV in NIH 3T3 cells transformed by human tumour DNA. 20 μ g of *Eco*RI-cleaved DNAs isolated from third-cycle transformants derived from rhabdomyosarcoma 1085 (a, b), lung carcinoma 1615 (c, d), pancreatic carcinoma 1189 (e, f), A2233 colon carcinoma cells (g), A427 lung carcinoma cells (h), A2182 lung carcinoma cells (i), A1604 gall bladder carcinoma cells (j) and A1698 bladder carcinoma cells (k) as well as from control NIH 3T3 cells (N), were blotted to nitrocellulose filters and hybridized to 4 $\times$ 10⁷ c.p.m. of nick-translated Kirsten-MSV DNA clones 4E *Pvu*II (ref. 25) or pHiHi-3 (ref. 16) as described in Fig. 1 legend. Co-electrophoresed DNA size standards were those described in Fig. 1 legend. Migration of the 6.8- and 3.0-kbp *Eco*RI-cleaved DNA fragments of the human *c-kis* gene¹⁶ is indicated by arrows.



labelled cell extracts of representative third-cycle transformants derived from each of the three human solid tumours and from several of the human tumour cell lines known to possess this gene (Table 2) were immunoprecipitated with antisera obtained from BALB-MSV-induced tumour-bearing rats. These antisera contain broadly reactive antibodies capable of recognizing not only the gene products of BALB-, Harvey- and Kirsten-MSVs, but also those coded for by the corresponding mouse and human homologue *c-onc* genes¹⁵. All the transformants tested exhibited elevated levels (about four- to eightfold higher than NIH 3T3 control cells as determined by densitometric analysis) of Kirsten-MSV p21-related proteins of molecular weights (MWs) 20,000–22,000 (Fig. 3). The different molecular weights exhibited by these proteins suggest the existence of some polymorphism in the human *c-kis* sequences. In one case (Fig. 3G), a third-cycle transformant derived from a pancreatic carcinoma exhibited an unusual Kirsten-MSV-related protein of MW 27,000. Because all other NIH 3T3 transformants derived from this tumour contained 21,000-MW proteins, it is possible that certain genetic alterations may occur during transfection. These results indicate that the observed Kirsten-MSV-related proteins represent the translational products of the transfected human oncogenes, thus providing further evidence that the *c-kis* sequences of this oncogene play a part in malignant transformation.

Previous studies have shown that a small percentage of human tumour cell lines contained oncogenes capable of inducing malignant transformation of NIH 3T3 mouse cells^{1–8}. We have demonstrated here that similar results can be obtained using solid human tumours. About 15% of the tumour specimens whose DNAs were tested in transfection assays were shown to contain dominant transforming genes. The relatively high transformation efficiencies observed with these solid human tumour DNAs implies the presence of oncogenes in most of the cells present in these tumours. These results support the hypothesis that these oncogenes have played a part in the development of these neoplasias.

Molecular characterization of dominant transforming genes present in 12 different human tumours has revealed the frequent detection of an oncogene containing sequences related to the *onc* gene of Kirsten-MSV. In fact, this oncogene was detected

in eight tumours, including a rhabdomyosarcoma and carcinomas of several organs such as colon, lung, gall bladder and urinary bladder. Other investigators have detected the same, or a closely related, oncogene in five additional lung and colon carcinoma cell lines^{4,5,9}. These findings, together with the recent demonstration that the T24 and EJ bladder carcinoma oncogene is a transforming allele of the normal human *c-has/bas* gene^{9,14,15}, have posed the question of why most of the transforming genes present in human carcinomas are related to the *c-has/bas/kis* gene family. It could be argued that

Table 1 Summary of DNAs isolated from naturally occurring human tumours tested for transformation of NIH 3T3 mouse cells in transfection assays

Type of tumour	Total tested	Positive in transfection assays
Carcinomas		
Bladder	2	0
Breast	2	0
Colon	2	2
Kidney	3	0
Lung	5	1
Ovary	4	0
Pancreas	2	1
Sarcomas		
Fibrosarcoma	4	0
Osteosarcoma	2	0
Rhabdomyosarcoma	2	1

Human tumours were thawed and the necrotic tissues removed with a scalpel. Tumorous tissue was finely minced with the aid of razor-sharp scissors, disrupted in a loose-fitted Dounce, washed three times with phosphate-buffered saline and incubated for 16 h at 37 °C with 200 µg ml⁻¹ of self-digested pronase in the presence of 0.2% SDS. High-molecular weight DNA was isolated by successive extractions with 1 vol of phenol, 1 vol of phenol/chloroform/isoamylalcohol (25:24:1) and chloroform/isoamylalcohol (24:1) followed by precipitation with 2 vols of ethanol. These DNAs (30 µg per plate, 16 plates in 4 different transfection experiments) were used to transfect NIH 3T3 cells by the calcium phosphate precipitation technique¹⁰, as described previously^{8,11}.

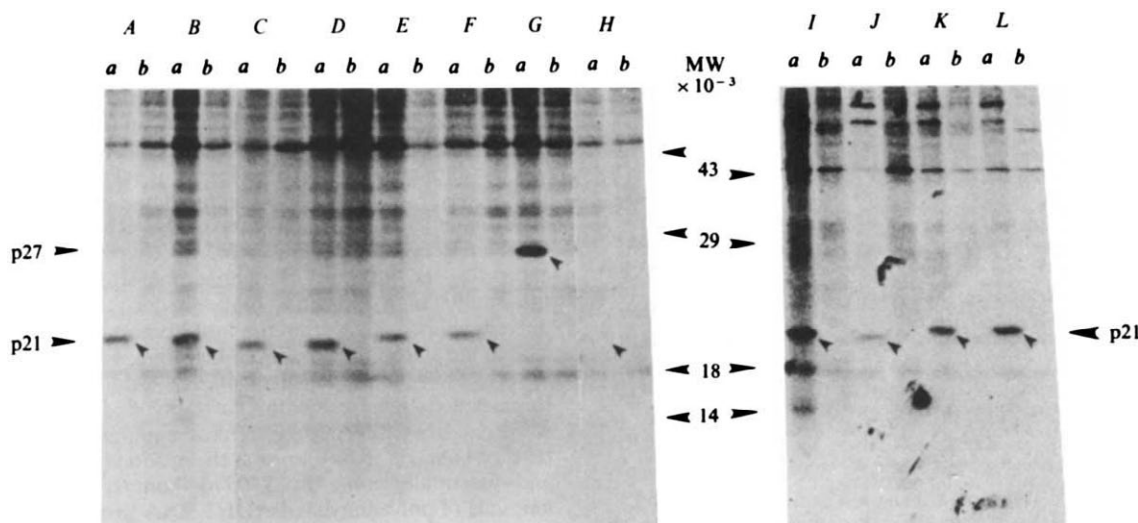


Fig. 3 Expression of proteins antigenically related to Kirsten-MSV p21 in NIH 3T3 cells transformed by human oncogenes known to contain *c-kis* sequences. Exponentially growing cells (about 2×10^6 cells per immunoprecipitation reaction) including third-cycle transformants derived from: A, B, lung carcinoma 1615; C, D, rhabdomyosarcoma 1085; E–G, pancreatic carcinoma 1189; H, control NIH 3T3 mouse cells; I, A2182 lung carcinoma cells; J, A1604 gall bladder carcinoma cells; K, A2233 colon carcinoma cells; and L, A1698 bladder carcinoma cells, were metabolically labelled with ³⁵S-methionine as previously described²⁶. Post-microsomal fractions were incubated for 1 h at 4 °C with 0.01 ml of sera obtained from: a, BALB-MSV-induced tumour-bearing rats¹⁵ or b, uninfected normal rats. Immunoprecipitates were collected with the aid of *Staphylococcus aureus* protein A bound to Sepharose 4B beads and disrupted in the presence of 1% SDS and 2% 2-mercaptoethanol by incubating them at 95 °C for 2 min. Denatured proteins were analysed by electrophoresis (18 h at 100 V) in SDS-polyacrylamide (14% w/v) gels. Electrophoresed gels were dried, fluorographed²⁷ and exposed to Kodak XR-5 films for 5 days at –70 °C. Co-electrophoresed molecular weight markers included ovalbumin (43,000), carbonic anhydrase (29,000), lactoglobulin (18,000) and lysozyme (14,000). Migration of proteins antigenically related to Kirsten-MSV p21 is indicated by arrows.

Table 2 Summary of human oncogenes detected in our laboratory

Type of tumour	Source of DNA	Ref.	Oncogene	Relationship with retroviral <i>onc</i> genes
Carcinomas				
Bladder	T24 cells	21	<i>onc</i> A	<i>has</i> , <i>bas</i>
Bladder	A1698 cells		<i>onc</i> B	<i>kis</i>
Colon	Solid tumour 1665		<i>onc</i> C	—
Colon	Solid tumour 2033		<i>onc</i> D	—
Colon	A2233 cells		<i>onc</i> B	<i>kis</i>
Gall bladder	A1604 cells		<i>onc</i> B	<i>kis</i>
Lung	A2182 cells	8	<i>onc</i> B	<i>kis</i>
Lung	A427 cells	22	<i>onc</i> B	<i>kis</i>
Lung	Solid tumour 1615		<i>onc</i> B	<i>kis</i>
Pancreas	Solid tumour 1189		<i>onc</i> B	<i>kis</i>
Sarcomas				
Fibrosarcoma	HT-1080 cells	23	<i>onc</i> E	—
Rhabdomyosarcoma	Solid tumour 1085		<i>onc</i> B	<i>kis</i>

Human tumour cell lines A1604, A1698, A2182 and A2233 were established by S.A.A. Their human origin and independent identity have been determined by karyologic and isoenzyme (ADA, G-6-PD, LDH, PGD, PGM₁ and PGM₂) analysis. *onc* A, *onc* B and so on is an arbitrary designation used solely to indicate genetically distinct oncogenes. Retroviral *onc* genes tested: *abl*, *bas*, *fes*, *mos*, *myb*, *myc*, *kis*, *sis* and *src*.

transfection protocols favour the identification of a particular type of human oncogene that preferentially transforms NIH 3T3 mouse cells. However, Lane *et al.*, using the same assay cells, have shown that none of nine oncogenes present in lymphoid human tumours appeared to be a member of the *has/bas* gene family⁹. Moreover, these authors have identified different transforming genes in neoplasias representing different stages of lymphocyte differentiation¹. Thus, it is possible that the *c-has/bas*-related oncogenes may be preferentially, although not specifically, activated in tumours of epithelial origin.

Shilo and Weinberg have recently shown that several 3-methylcholanthrene-transformed mouse cell lines may contain the same oncogene¹⁹. Interestingly, all oncogenes so far detected in carcinomas of the lung appeared to be the same (Table 2; refs 5, 6, 9). Although no definitive aetiology has been established for any form of human cancer, most lung carcinomas have been associated with smoking²⁰. These observations are consistent with the hypothesis that neoplasms induced by certain carcinogens may be associated with the activation of a particular cellular oncogene.

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Regulatory mutants of the maize *Adh1* gene caused by DNA insertions

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Transposable elements in maize can induce changes in the timing of gene expression and the quantity of gene product^{1,2}. The timing of expression of the *bronze* locus has been shown genetically to be affected by insertion of the transposable element *Ds* (dissociation)³. Molecular evidence has demonstrated that insertion of *Ds* at another locus, *shrunk-1*, disrupts the DNA sequence as expected of an insertion^{4,5}. Here we report quantitative changes in gene expression resulting from the insertion of a 1.5-kilobase (kb) DNA element at an affected gene. The alcohol dehydrogenase-1 (*Adh1*) mutants described here, selected via allyl alcohol-resistant pollen grains⁶, were recovered from a genetic background containing Robertson's mutator (*Mu*)⁷. We suggest that the *Mu* genetic background caused the DNA insertion at *Adh1*, and that the insertion assumed some regulatory function in the control of *Adh1* expression.

The *Adh1* alleles considered here, and their products, have been described elsewhere^{8,9}. Their genealogies and characteristics are summarized in Fig. 1. *Adh1-S3034* (*S3034*) was induced in an *Adh1-S* (*S*) progenitor allele as a consequence of crossing an *S* line to a line carrying *Mu* and an electrophoretically distinct *Adh1* allele. The variant *Adh1* in the *Mu* line allowed unambiguous identification of *S* alleles among progeny, and recovery of marker genes from both parental lines ensured that *S3034* was not the product of pollen contamination.

S3034 is expressed in all organs normally exhibiting the *S* gene product, but at a level 40% that of the progenitor. It is 100-fold less stable than ethylmethane sulphonate (EMS)-induced mutants as judged from pollen samples stained for alcohol dehydrogenase (ADH) activity. Genetically unstable derivative alleles having even less ADH1 were selected from *S3034* lines as shown in Fig. 1: *S3034b* expresses 13% of the ADH1 activity seen in *S*, and *S3034a* expresses none.

ADH1 polypeptides are synthesized *de novo* by roots in response to anaerobic stress^{10,11}. Gerlach *et al.*¹² have demonstrated, and we have confirmed in our lines (data not shown), that increased levels of ADH1 produced in anaerobic conditions are accompanied by increased levels of ADH1 RNA. Steady-state RNA levels are reached within 6 h.

We compared polyadenylated ADH RNA levels in roots from homozygotes of *S*, *S3034*, *S3034a* and *S3034b* after subjecting seedlings to 12 h of anaerobic stress. Figure 2 shows the results of a Northern blot in which equal amounts of polyadenylated RNA from the four homozygotes were hybridized to the cDNA probe pZmL84 (see Fig. 4 legend for descriptions of the probes). Results from RNA dot-blots of the same samples are shown below the corresponding gel tracks. 'Dots' were cut from filters for Cerenkov counting: ~40% normal levels of ADH1 RNA were seen in *S3034*, 15% in *S3034b* and essentially none in *S3034a* homozygotes. Thus, the amounts of polyadenylated ADH1 RNA produced by the progenitor and mutant alleles reflect relative enzymatic activities in the induced roots.

Decreased levels of ADH1 RNA in the mutants are not due to a general failure of anaerobic metabolism. Seedlings homozygous for either the mutant alleles described here or any of several *Adh1*-null alleles (which have no ADH1 polypeptides) continue to support protein synthesis for over 12 h. Indeed, several null mutants produce normal levels of ADH1 RNA for

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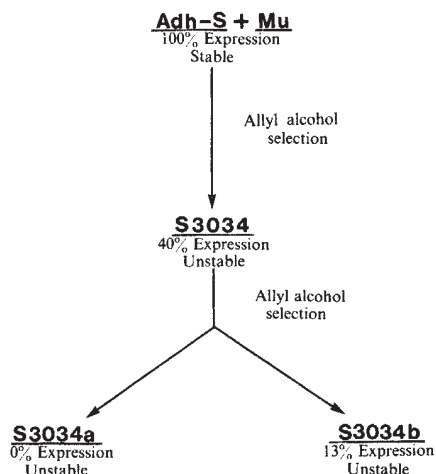


Fig. 1 The origin of our four *Adh1* alleles and a summary of their characteristics⁹. The *S* parent carried an electrophoretically identifiable EMS-induced mutant of *Adh2* which was recovered in *S3034* progeny, as was the somatic variegation phenotype characteristic of mutator lines. We have no lines capable of producing the *S3034* phenotype due to contamination. The *S* allele is defined as stable and 100% expressed. ADH1 activity was measured in mature scutella, in primary roots subjected to 24 h of anaerobic stress, and in pollen grains. Stable denotes (1) a phenotypic pollen revertant frequency similar to that of EMS-induced mutants at *Adh1-S*, that is, 2×10^{-6} ADH⁺ pollen per total pollen¹⁶; and (2) a forward frequency of 2×10^{-7} ADH⁺ viable pollen per total viable pollen¹⁷. Reversion frequencies of mutant alleles are $1-2 \times 10^{-4}$ ADH⁺ pollen per total pollen. Cytochemical staining of pollen or of germinating pollen *in situ* for ADH activity generated the data used to measure genetic stability.

that period (M. Sachs, personal communication and S.H., data not shown). Further, cDNA probes for other anaerobically induced RNA species detect the same levels of these other RNA species in all four genotypes (S.H., data not shown). We conclude that our four *Adh1* alleles differ in their quantitative expression because their ADH1 RNA levels differ, and that the observed changes are not merely secondary effects.

Figure 3 depicts Southern blots of genomic DNA probed with ADH1 cDNA from the plasmids pZmL84 and pKM1 (see Fig. 4 for descriptions of probes). Three conclusions can be drawn from the results. First, our hybridization conditions allow the cDNA to recognize a unique sequence of maize DNA. Second, in several cases *S3034* and its derivatives reside on fragments ~1.5 kb longer than fragments containing the progenitor allele. Third, despite the variations in ADH1 RNA levels, there are no detectable differences in endonuclease recognition sites around *S3034*, *S3034a* and *S3034b*.

A variety of single- and double-enzyme digests of genomic DNA allowed construction of the maps given in Fig. 4. Assignment of transcriptional polarity was simplified by the presence of a *Hind*III site in the *S* structural gene. Because the cDNA of pZmL84 has been sequenced¹² and correlated with peptide sequences (Inglis and Peacock, personal communication, and Kelly and M.F., unpublished data), there was no ambiguity in the assignment of polarity. The *Hind*III site in the recombinant clones allowed us to hybridize Southern blots of *Hind*III-digested DNA with 5'- and 3'-specific probes. Figure 4 insert illustrates the results of such an experiment. Clearly, the mutant *S3034* and its derivatives contain ~1.5 kb of additional DNA. This DNA element, containing a *Bst*EII recognition site, lies upstream from the ADH1-coding sequence represented in a probe which is 90% full-length (pKM1).

Although we have not proved that the *Mu* genetic background caused the DNA insertion at *Adh1*, evidence supports this hypothesis. Robertson's *Mu* background was crossed into the genotype from which the *S3034* pollen was selected. The pollen parent of *S3034* exhibited the behaviour characteristic of a *Mu* background⁷, and the progeny exhibited the somatic

variegation phenotype typical of *Mu* and heritable as a dominant non-mendelian trait (M.F. and J.N.S., to be published elsewhere). Furthermore, two additional *Mu*-induced mutants of *S* have been recovered, and they too contain 1.5 kb inserted DNA (R. Johns and J.N.S., unpublished data). Therefore, it seems most unlikely that the insertion of DNA at *S* occurred independently of *Mu*. However, we cannot conclude that the inserted sequences are Robertson's *Mu*. A molecular probe will be required to define *Mu* itself.

Evidence suggests that the quantitative regulation of *Adh1* by inserted DNA sequences occurs at the level of transcription or processing. The length of mature ADH1 RNA is unchanged, and the polypeptide produced by the mutants is unchanged in length, charge and specific activity⁹. We cannot exclude the possibility that less ADH1 RNA is seen in the mutants because it is more rapidly turned over, but our data are most compatible with altered transcription or processing.

The presence of inserted elements in *S3034a* and *S3034b* is not surprising, because these derivatives remain genetically unstable (Fig. 1). The lack of detectable differences between *S3034* and its derivatives was unexpected: transposition-associated events mapped at the DNA sequence level in other organisms involve conspicuous changes¹³⁻¹⁵. Although independent events occurring beyond the 20-kb window provided by our Southern blots might be responsible for decreases in expression of *S3034a* and *S3034b*, the distance required and the severity of the effect render this explanation unlikely. Alternatively, the unstable derivatives may differ from *S3034* by minor sequence changes that are below our threshold of detectability.

A possible interpretation of our results is that a *Mu* insertion has taken over some aspect of the regulation of *Adh1* expression. *Adh1-S* is expressed only in certain tissues; it is inducible by anaerobic stress and by the plant growth hormone, 2,4-dichlorophenoxyacetic acid⁶. Furthermore, we know that DNA acting in *cis* to *S* affects quantitative expression in an

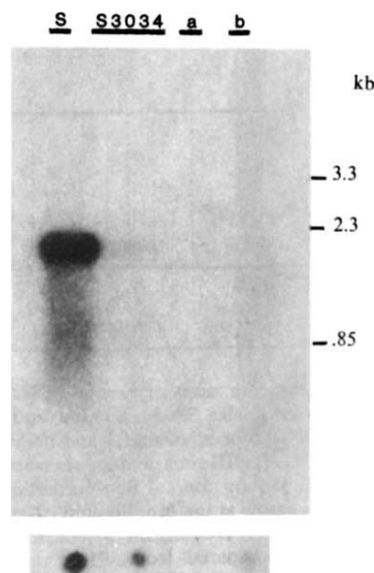


Fig. 2 Autoradiograms of Northern blots of polyadenylated RNA probed with the ADH1 cDNA plasmid pZmL84 (ref. 12) (see Fig. 4 legend for description of probes). Maize kernels of defined *Adh1* genotype were germinated and seedlings grown until primary roots were 5–10 cm long, at which time the seedlings were immersed in weakly buffered water for 12 h. Total RNA was isolated by the procedure of Lizardi and Engelberg¹⁸. We define polyadenylated RNA as that portion of total RNA recovered after selective binding to oligo(dT)-cellulose (T-2; Collaborative Research). The gel illustrated contained glyoxylated RNA (5 µg per track), separated on an agarose gel¹⁹ and transferred to DPT paper by the unpublished procedure of B. Seed. Hybridizations were as described elsewhere¹⁹ at 42 °C for 24 h with the addition of 100 µg ml⁻¹ polyadenosine; washes were as described by Gerlach *et al.*¹². From left to right, polyadenylated RNA from *S*, *S3034*, *S3034a* (*a*) and *S3034b* (*b*) homozygotes.

Fig. 3 Autoradiograms of probed Southern blots comparing the progenitor *S* allele with mutants *S3034*, *S3034a* and *S3034b*, and revealing the presence of ~1.5 kb additional DNA beyond the region of probe homology. Tracks are grouped by the endonuclease used to digest the genomic DNA before electrophoresis; *S*, *S3034a* and *S3034b* are displayed from left to right within each set. Genomic DNA was prepared by the method of Murray and Thompson²⁰, digested with restriction endonucleases as recommended by the supplier (BRL) and fractionated with 15 µg per track on 0.7% agarose (Seakem) gels in 90 mM Tris-base, 90 mM H₃BO₃, 3 mM EDTA, pH 8.3. Plasmid DNA was prepared by CsCl banding of cleared-lysate DNA²¹ which had been precipitated with polyethylene glycol, and inserted cDNA sequences used as probes were isolated electrophoretically from plasmid endonuclease digests. Nick-translations, Southern blots and hybridizations were done using the procedure of Engel and Dodgson²² with washing in 0.2×SSC, 0.1% SDS at 68 °C.

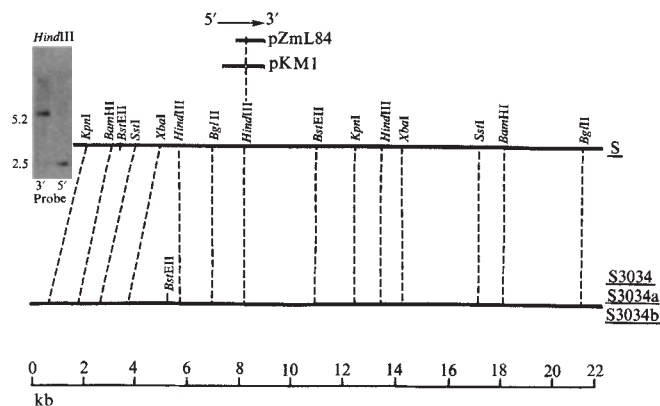
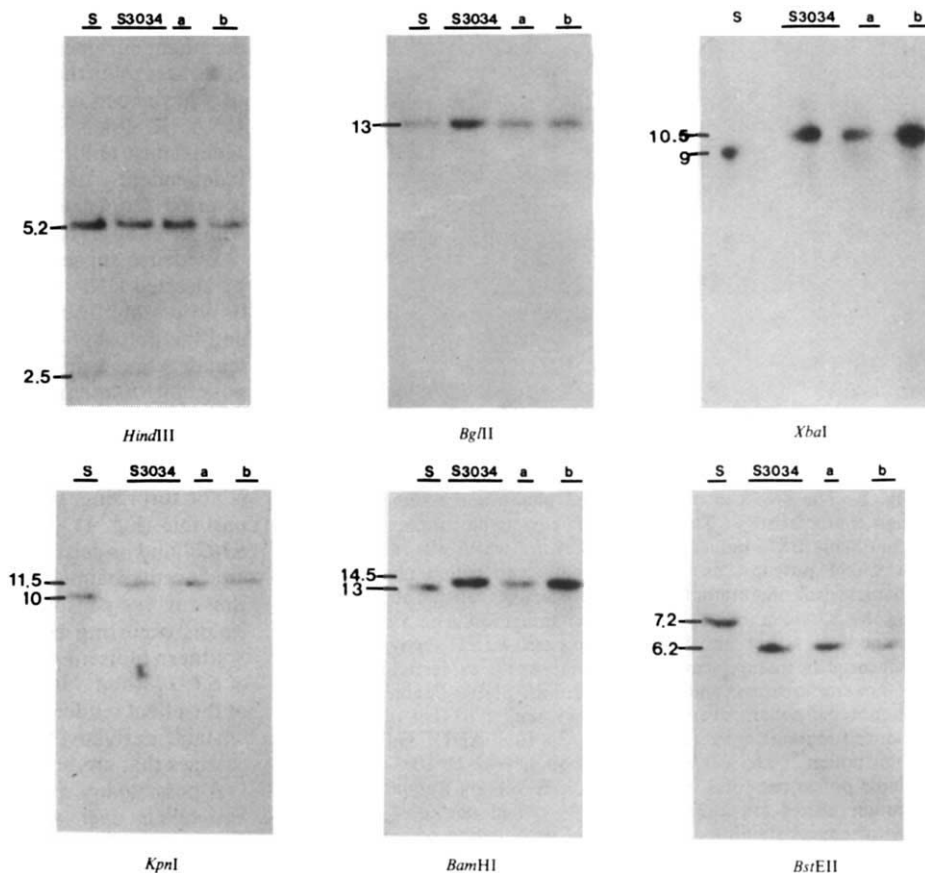


Fig. 4 Endonuclease site maps of genomic DNA containing *S* (top) and the mutant alleles *S3034*, *S3034a* and *S3034b* (bottom). The two cDNA probes, pZmL84 and pKM1, are aligned by their homologous *Hind*III sites, and the shortest possible transcriptional unit accounting for a 1,650-nucleotide transcript is represented by the arrow at the top. Plasmid pZmL84 was given by Gerlach *et al.*¹² and represents the 3' half of *ADH1* mRNA. Plasmid pKM1 was prepared from cDNA to anaerobically-induced mRNA, cloned in pBR322, detected as an anaerobically-stimulated sequence, and identified as *ADH1* cDNA by hybridization to pZmL84; it contains ~1,500 base pairs (bp) of coding sequence. Localization of each endonuclease digestion site was confirmed with at least two sets of two-enzyme digests. Maps of *S3034*, *S3034a* and *S3034b* were generated independently. The inset illustrates the Southern blot of an *S* *Hind*III digest used to determine transcriptional orientation of the gene. The cDNA from pZmL84, with ~200 bp 5' and 700 bp 3' to the internal *Hind*III site, hybridized primarily to the large (5.2 kb) genomic fragment produced by *Hind*III digestion of *S*, while excised pKM1 sequences representing a region 5' to the *Hind*III site recognized only the short (2.5 kb) genomic fragment. Note that the *Bst*EII site seen 5' to the hybridizing sequences in *Adh1-S* is presumed present in *S3034* and its derivatives but cannot be detected due to the appearance of a new *Bst*EII site closer to the region of probe homology.



organ-specific way⁹. *S3034* and *S3034b* continue to exhibit all these aspects of normal regulation, producing 40% and 13%, respectively, of the level of *ADH1* synthesized by *S*; organ specificity, developmental timing and inducibility remain unaltered. If *Mu* has assumed a regulatory function in the control of *Adh1-S* expression, it would seem to be a simple quantitative function that has been usurped.

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Antibodies to Z-DNA interact with form V DNA

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Annealing of single-stranded (ss) DNA rings of complementary base sequence gives rise to the so-called form V DNA which is characterized by a linking number of zero¹. The similarity of the circular dichroism spectrum of form V DNA¹ with that of the 1:1 mixture of the low salt (B-type) and the high salt (Z-type) form of poly(dG-dC)·poly(dG-dC)^{2,3} suggests that form V DNA might contain left-handed double-helical DNA of the Z-type. The discovery that the left-handed Z-form of poly(dG-dC)·poly(dG-dC) induces a strong immune response^{4,5} has provided a novel means of searching for DNA of this particular conformation⁶. We therefore used antisera and monoclonal antibodies to Z-DNA to determine whether form V DNA contains stretches of left-handed double-helical conformation. We report here that form V DNA competes in the binding of antibodies to Z-form poly(dG-dC)·poly(dG-dC). The competition by form V DNA is similar to that of (dG-dC)₅·(dG-dC)₅. Thus, DNA of natural base sequence, when forced to adopt left-handed conformations by topological restrictions, is recognized by antibodies against Z-DNA.

Form V DNA, an 'unlinked' covalently closed, circular double-stranded (ds) molecule, is a form of DNA in which any right-handed double-helical structure has to be compensated by negative supercoils and left-handed duplex turns. It adopts a tightly folded structure, as revealed by sedimentation, electron microscopy and gel electrophoresis¹. To date, it is the only form of DNA having a natural base sequence that displays a negative circular dichroism at 290 nm (Fig. 1). Such a negative circular dichroism band has been observed previously for poly(dG-dC)·poly(dG-dC) at high salt or ethanol concentration^{2,3} and is characteristic of the left-handed double-helical conformation of Z-DNA⁷.

Antisera against Z-DNA were obtained from a New Zealand White rabbit and BALB/c mice, respectively, after immunization with brominated poly(dG-dC)·poly(dG-dC), as described by Lafer *et al.*⁴. Such a mouse was used also for the selection of hybridomas producing monoclonal antibodies against Z-DNA (R.T., M. Leptin and F.M.P., in preparation). As the preparation of labelled form V DNA is very difficult, the interaction with antibodies was studied by the displacement of radioactively labelled poly(dG-dC)·poly(dG-dC); Fig. 2 summarizes the results of radioimmuno-competition assays in conditions in which poly(dG-dC)·poly(dG-dC) adopts the left-handed Z-form^{2,7}.

In the experimentally accessible range of concentrations, form V DNA of plasmid pBRβG2.17 competed with poly(dG-dC)·poly(dG-dC), whereas no competition was observed for open circular or covalently closed circular DNA with a natural superhelical density. No competition with circular ssDNA from phage M13 was found. This observation using rabbit antiserum has been substantiated with a purified monoclonal antibody against Z-DNA (Fig. 2a).

The concentration of form V DNA necessary for 50% inhibition of binding of the labelled polymer was about 400 times higher than obtained with unlabelled poly(dG-dC)·poly(dG-dC); this may be due either to much weaker binding of the antibodies to this DNA and/or to a low amount of Z-like DNA in form

V. Figure 2b shows that the competition by oligo(dG-dC)·oligo(dG-dC) with a chain length of 10–14 base pairs (bp) is very similar to that of form V DNA. Poly d(I-5BrC)·poly d(I-5BrC), which also gives an inversion of the circular dichroism spectrum in high salt concentrations⁸, competes even less and its effect is similar to that of (dG-dC)₄·(dG-dC)₄. The considerably stronger interaction with the polymeric antigen may be due to additional stabilization of the complex by the second binding site on the antibody compared with the short oligonucleotides, which apparently act as haptens.

To determine whether form V DNA interacts with antibodies at lower salt concentrations, the following more direct, qualitative approach was chosen (see Fig. 3): form V DNA was incubated with different amounts of antibodies to Z-DNA in 0.2 M NaCl, the reaction mixture applied to an agarose gel, and the DNA visualized by the fluorescence of bound ethidium bromide. The antibody–DNA complex does not enter the gel or is considerably retarded. Comparable results were obtained also with form V DNA prepared from phage PM2 (data not shown). The small amount of open circular DNA served as an internal standard, as it does not interact with the antiserum. For this type of assay, the final purification of form V DNA is unnecessary, as the antibodies discriminate between underwound closed circular DNA and the other forms of DNA.

The total amount of antibody necessary to retard 50% of the form V DNA could be estimated only very roughly and seems to be about half that necessary to precipitate the equivalent amount of poly(dG-dC)·poly(dG-dC) in 4 M NaCl. These results indicate that a considerable part of form V DNA interacts with antibodies to Z-DNA. The sequence of the plasmid pBRβG2.17 contains as alternating dG-dC stretches, one sequence of six base pairs and seven of five base pairs. If one considers also alternating purine–pyrimidine stretches, the lon-

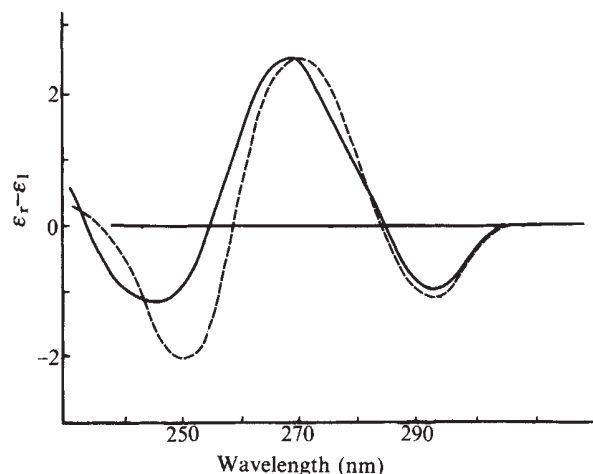


Fig. 1 Circular dichroism (CD) spectrum of form V DNA of pBRβG2.17 in 10 mM Tris-HCl pH 7.9, 1 mM EDTA (—) and a mixture of 50% low salt form (B-type) and 50% high salt form (Z-type) of poly(dG-dC)·poly(dG-dC) in 2.3 M NaCl, 10 mM Tris-HCl pH 7.9, 1 mM EDTA (---) as measured in a Cary 60 spectropolarimeter with CD attachment. The concentration of DNA was determined from the absorbance at 260 nm, using $6.8 \text{ mM}^{-1} \text{ cm}^{-1}$ for form V DNA and $7.2 \text{ mM}^{-1} \text{ cm}^{-1}$ for the low salt form of poly(dG-dC)·poly(dG-dC)². Form V DNA was prepared by nicking closed circular DNA of the plasmid pBRβG2.17 with DNase I. Circular ssDNA was enriched by alkaline sucrose gradient centrifugation, brought to pH 9.0 and precipitated with ethanol. It was further purified by neutral sucrose gradient centrifugation as described by Stettler *et al.*¹. We estimate that more than 95% of the DNA is form V, as judged in gel electrophoresis. Electron microscopy revealed highly folded molecules. Form V DNA could be cleaved with restriction endonuclease *Cfo*I and the fluorescence in $2.5 \mu\text{M}$ ethidium bromide pH 8.0 was almost the same before and after digestion, in contrast to naturally supercoiled form I DNA. $\epsilon_r - \epsilon_l$, difference of extinction coefficients for right and left circular polarized light ($\text{M}^{-1} \text{ cm}^{-1}$).

gest is 10 bp (Ph. Bucher, personal communication). Thus, it is tempting to conclude that a left-handed DNA conformation is not restricted to such a type of sequence as deduced from the X-ray structure determination of oligo(dC-dG)·oligo(dC-dG)^{9,10}. This was the conclusion also of a study of the optical properties of form V DNA (S. Brahms *et al.*, in preparation).

Whether the apparently weaker interaction of our antisera with form V DNA than with the Z-form of poly(dG-dC)·poly(dG-dC) is due to subtle differences in the conformation of

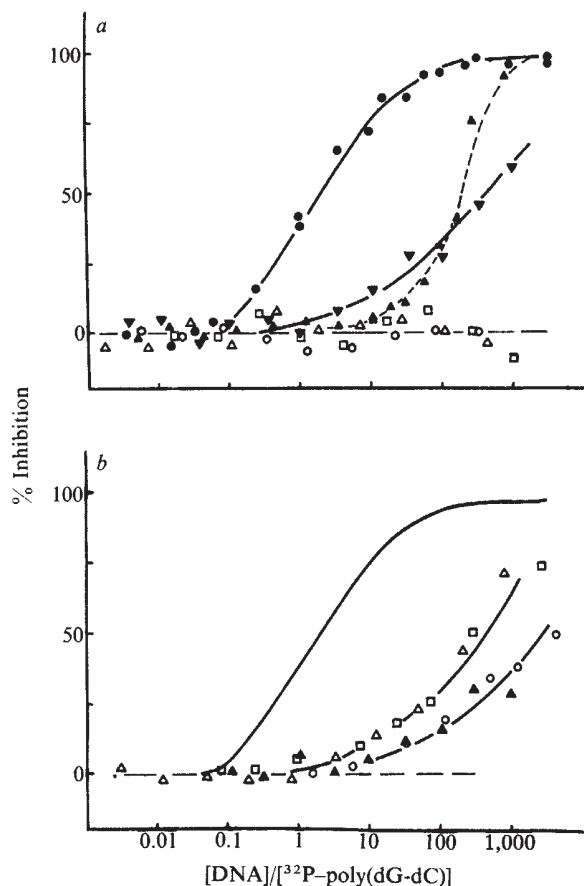


Fig. 2 Radioimmuno-competition assay with ³²P-labelled poly(dG-dC)·poly(dG-dC) in 4 M NaCl. *a*, Inhibition of the binding of radioactive poly(dG-dC)·poly(dG-dC) to rabbit antiserum by unlabelled poly(dG-dC)·poly(dG-dC) (●), and form V DNA (▲), prepared from the plasmid pBRβG2.17 (4,961 bp) according to Stettler *et al.*¹. No inhibition is found with open circular (○) and superhelical, closed circular DNA (△) from the same plasmid, or with ssDNA prepared from phage M13 (□). ▼, A purified monoclonal antibody, Z-D11, and form V DNA were used in otherwise identical conditions. *b*, Inhibition by (dG-dC)_n·(dG-dC)_n of different chain lengths (○, 8; □, 10; △, 14 bp) prepared as described previously¹², and poly(dG-dC)·poly(dG-dC) (—) (see *a*); ▲, inhibition by poly d(I-5BrC)·poly d(I-5BrC) (PL Biochemicals). Poly(dG-dC)·poly(dG-dC) was synthesized as described previously² and the polymer was end-labelled with [α-³²P]dCTP (3,000 Ci mmol⁻¹) and DNA polymerase I from *Escherichia coli* to >5,000 c.p.m. ng⁻¹. Serum against Z-DNA was obtained from a rabbit immunized with brominated poly(dG-dC)·poly(dG-dC) according to Lafer *et al.*⁴. A given amount of DNA was incubated with 3.5–5 ng ³²P-labelled poly(dG-dC)·poly(dG-dC) and 0.05–0.07 μl rabbit antiserum or 14 ng purified monoclonal antibody in 100 μl solution (4 M NaCl, 60 mM sodium phosphate, 30 mM EDTA pH 8.0) for 1 h at room temperature. Goat antiserum to rabbit or mouse immunoglobulins was added, the mixture kept for another hour at room temperature, and then centrifuged. The radioactivity of the supernatant and washed pellet, respectively, was determined by Cherenkov counting. In the absence of competing DNA 30–60%, and using serum from a non-immunized rabbit, less than 0.5% of the radioactivity was found in the pellet.

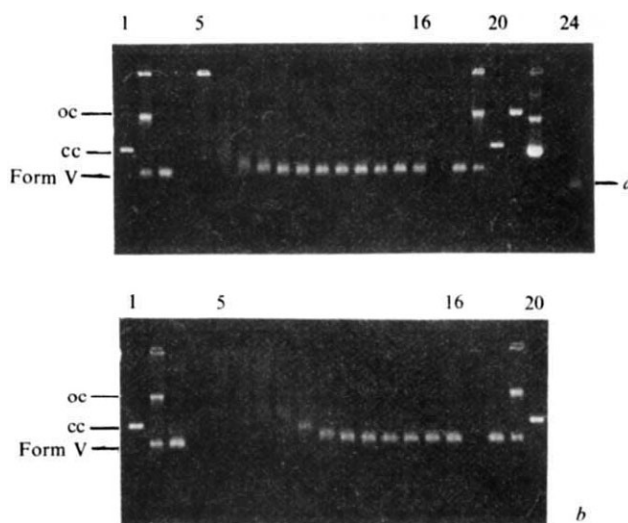


Fig. 3 Electrophoresis of form V DNA of plasmid pBRβG2.17 after reaction with different amounts of antibodies to Z-DNA in serial 1:2 dilution (lanes 5–16): starting with *a*, 1.3 μl rabbit antiserum; *b*, 2 μg monoclonal antibody Z-D11. Form V DNA (115 ng) was incubated for 1 h at room temperature with the indicated amount of antibody in a total volume of 10 μl (0.2 M NaCl, 60 mM sodium phosphate, 30 mM EDTA pH 8.0), 2.5 μl 50% glycerol added, and the solution loaded on to a 1% agarose gel. The electrophoresis buffer contained 36 mM Tris, 30 mM NaH₂PO₄, 1 mM EDTA, 1.25 μM ethidium bromide. Electrophoresis was at 5 V cm⁻¹ for ~2 h and the gels were photographed under UV illumination. Control sera showed no effect and the following DNA samples were included as references: lanes 1, 20, natural closed circular (cc) DNA; lanes 2, 19, form V DNA before neutral sucrose gradient centrifugation with open circular (oc) and 'unlinked' closed circular DNA; lanes 3, 18, form V DNA without antibody; 21, open circular DNA; 22, pBR322 DNA; 24, form V DNA denatured in 0.2 M NaOH.

left-handed 'natural' DNA compared with that of poly(dG-dC)·poly(dG-dC), or to the lack of cooperation between the two binding sites on an antibody molecule, to structural perturbations at junctions between right- and left-handed segments¹¹, or to some other reason is unknown at present.

We thank Professor Theo Koller for encouragement and helpful discussions, the Schweizer Nationalfonds zur Förderung der wissenschaftlichen Forschung (Professor Th. Koller), and the Deutsche Forschungsgemeinschaft (Po 155/6) for financial support.

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Corrigendum

In the letter 'Redesigning enzyme structure by site-directed mutagenesis: tyrosyl tRNA synthetase and ATP binding' by G. Winter *et al.*, *Nature* **299**, 756–758 (1982), 'lower *K_m*' in the last line of the introductory paragraph should read 'poorer *K_m*'.

CHRISTMAS BOOKS SUPPLEMENT

Where do rainbows come from?

Peter Newmark looks at some of the science books for children recently published in Britain*.

WHAT an odd and lopsided view of science children would be left with from this selection of books. For the younger age groups the emphasis is on natural history and dinosaurs with a modicum of computers and a sprinkling of the four elements. For children approaching the end of their first decade the tables are turned: computers and other artefacts of high technology take first place from natural history and fossils, with some elementary physics thrown in. But, for whatever the age, of physiology, chemistry and genetics there is seldom a word. Even within the limited range of topics that seem to be considered palatable to the customer there are many missed opportunities for slipping in a scientific concept or two, surreptitiously if needs be.

Under-6s

It is a pity, for example, that Althea Braithwaite did not introduce the concept of photosynthesis — but not the word — in *Tree* (£2.95), one of Longman's *Life Cycle Books*. The tree in question, an oak, is said to obtain food and water through its roots, and to take back food from the leaves before they fall. When on the tree the role of dead leaves is, we are told, to protect new buds, and when off it to protect fallen acorns; but the living leaves are never given any function. Equally it would not have been impossible to have hinted at the concept of inheritance or of territory in *Foxes* (£2.95), another of the same series. That apart, both books are blessed with bright and uncluttered illustrations and — how nice to see them — delightful endplates.

Much more sophisticated illustrations adorn Vanessa Luff's *The River* (A&C Black, £3.95), 12 double-page spreads depicting the course of a river from its moorland source to the sea. Each spread is of a different habitat with representative fauna and flora, and carries only a nominal sentence or two of text. There is, however, an identification key to each picture at the back of the book. *The River* is for parent and child to "read" together with much adult pleasure to be had from the illustrations. There is rather less in the way of adult entertainment in either *Frogs* or *Butterflies* (Heinemann/Quixote Press, £2.95 each; to be published in the United

States by Putnam at \$6.95), two rather crudely illustrated Natural Pop-Ups. However the pop-up pictures and other paper mechanics work quite well and whisk one through the life-cycle of the beast concerned in all of five double pages. Your infant prodigy might notice that frogs mate before the female produces eggs whereas the female butterfly appears to go in for virgin birth.

6-8-year-olds

So, too, does — or rather did — the brontosaurus, if I am not being misled by no less an authority than Beverly Halstead. My impression is gained from *A Brontosaurus: The Life Story Unearthed* (Collins, £3.95), illustrated by Jenny Halstead and written by Beverly. In its 15 double-page spreads is recounted the 120-year life of a brontosaurus named Ajax, whose mate lays eggs every spring as though that were the sole meaning of coming into season. The story is nicely illustrated and well told in about 100 words per page. Cleverly, there is also a boxed sentence or two per page which explains the evidence on which the depicted scene has been reconstructed.

Passing quickly over *Space Mission* and *Underwater Mission* by Terry Pastor (Methuen-Walker, £3.95 each), pop-up books that are more gimmick than value, brings me to four topic books from the same publishing stable: two of five in the *Animal Lives* series and the first two of *The Elements*. The animal lives are those of *The Spider* and *The Fox* (£3.95 each; published in the United States by Dial, \$9.95 each). Both books are written by Margaret Lane, biographer of Beatrix Potter. Those whose sympathy is with the uneatable rather than the unspeakable in the fox hunt should be warned that Margaret Lane's views of the chase are that "It is not an easy sport, for the fox has his own methods of escape"; and anyone with a predisposition to arachnophobia had better be spared Barbara Firth's larger-than-life illustrations in *The Spider*. Both her illustrations and those by Kenneth Lilly for *The Fox* are stunning. David Lloyd's *Air*

(£3.95) — one of *The Elements* books — is also well illustrated with a text that is informative but dull; in *Fire*, by John Satchwell (£3.95), the text outdoes the illustrations. All four books are well worth giving to the child who has started to investigate and write about particular topics at school — although sod's law being what it is, the chosen topic is bound never to coincide with the books available.

A much more unusual and altogether inspired book, the first in the *Tell Me About...* series, is *The Earth and the Sky* (Collins, £3.50) which has been translated from a French text by Pierre Avérous. It takes the form of about 75 questions and answers, three to the double page, on the weather, the Earth and the sky. Not only do they help the child to understand but also the adult to explain how, for example, night comes, where rainbows come from and why one cannot see that the Earth is round. Clever and inventive answers are given in both words and illustrations; the book is a Godsend for the harassed parent.

8-11-year-olds

There is also an inspired and out of the ordinary book for older children in Peter Ward's *The Adventures of Charles Darwin* (Cambridge University Press; £3.75, \$7.95). George Carter, a cabin-boy on the *Beagle*, befriends Mr Darwin who proceeds to share his thoughts on such matters as slavery, the presence of sea shells on mountain tops, the variety of finches on the Galapagos Islands and much else besides. The educative role is never obtrusive, being carefully placed among adventures with South American gauchos and under Argentinian gunfire. Finely illustrated, and with chapter headings such as "Mr Darwin gets Ants in his Pants", this is writing for children at its best.

Dinosaurs by David Lambert (Kingfisher/Crown; £5.95, \$6.95) is a much more conventional book but excellent in its own way. Its 90 large pages have vivid illustrations, graphics and photographs which provide a good counter-balance to the rather academic text. Complete with glossary and index, large-lizard-lovers will find this a nice book for browsing and better still for reference.

For reference sake, perhaps to coincide with a holiday, Penny Anderson's *The*

*Where possible, details of availability and price in the United States are given. All readers should check prices before ordering.

Wildlife of Mountains and Moorlands (Macdonald, £3.95) — one of a series of six *Nature in Focus* books — can also be recommended. Sixteen well-illustrated double-page spreads cover the designated habitat, its fauna and flora and its changes with the seasons. Useful for the purposes of identification, the scientific titbits are a welcome bonus. Almost at the other end of the scale is **Animals of the Mountains and Forests** (Burke; £4.95, \$12.95) and its five companion volumes in the series *Animals and their Environment*. The choice of topics to occupy the 32 double pages appears to be far too arbitrary and the whole approach rather incoherent. Why, for instance, devote two whole pages to the seven-spot ladybird and include several species that are as typical of suburbia as of the habitat of the title? A redeeming feature of the books is their use of impressive colour photographs wherever possible.

Inanimate in subject but not in style is **Earthquakes and Volcanoes** by Sara Steel (A&C Black, £3.95), one of this publisher's Junior Reference Books. It starts with 10 pages on the causes of earthquakes and volcanoes and then devotes 50 more to descriptions of some of the most famous, from Santorini to Mount St Helens. Full of dramatic photographs and gory detail, this will be a sure-fire success as a topic book. It is more of a puzzle to know what to make of **Sam's System: A Guide to Computers** (Dent, £3.95), in which Rosemary Court adopts the anthropomorphic approach:

"... it had two little feet dangling over the edge of the desk, two hands folded neatly under the screen . . . I'm TAK the terminal. TAK's my pet name — but I have other names too. VDU and CRT, for instance". By chatting to TAK the terminal, Sid Software, Susie the Supermarket Stock Control Program and a host of others, Sam (of the title) becomes wise beyond his years. I could not stomach this book, but buy it if you are intent upon training a tiny technocrat.

A much more conventional approach is taken by Gareth Renowden and Nigel Hawkes in **Video and Space Shuttle**, respectively (Collins/Gloucester Press; £2.95, \$9.40), in the *Inside Story* series. Each has 30 large pages, with illustrations taking more space than text. There are diagrams, cut-aways, a little technical data, a glossary and an index but in the end too much knowledge is assumed and too little information provided. Nevertheless these are attractive books of some value.

For something completely different your child might like to learn that 'flu viruses from outer space can result in the spread of epidemics on Earth. It's Fred Hoyle again but here in combination with his son Geoffrey and with the decency for the sake

of children to wrap his proposals firmly in the cloth of science fiction. **The Planet of Death** is one of four of their science fiction books, published by Ladybird at 60 pence (\$1.95) apiece, and all good yarns. *The Planet of Death* is typical: William, a good schoolboy, has considerably less time for the vivacious Kiryl than for her father, Professor Gamma, who is adept at the art of dematerialization when it is necessary for our three heroes to visit another planet.

11-year-olds and over

There are both books of facts and books of experiments for this age group, the former including three *Granada Guides on Fossils, Computers and Robots* (£1.95 each). Pocket-sized, with glossary, index and many illustrations, children will find

the case of **How to Make Square Eggs** by Paul Temple and Ralph Levinson (Beaver, £0.95), the title experiment worked but I cannot guarantee equal success for the square tomato (start with a seed and encase an infant fruit in a box) or for the experiment entitled "A plant that eats animals" (first buy a Venus fly trap). A paperback, with 40 experiments rated by difficulty and some aimed for the classroom rather than home, it would engender both amusement and frustration. Leonard Marsh's **The Guinness Book for Young Scientists** (GBR Educational, £2.95) is better in that most of the experiments use household materials, although very basic chemistry apparatus is required for some and a trip to the chemist for others. Experiments include how to make plastic from milk and hydrogen sulphide from a metal bottle cap!

Like the other books from Methuen-Walker, **Amazing Air** and **Supermotion** (£3.95 each), two of the *Science Club* series, are beautifully designed and illustrated. There are about 30 experiments in each book, carefully explained and illustrated, step by step, with attention to detail, supplies and safety. *Amazing Air* has experiments on such phenomena as air pressure, oxidation and electrolysis; spinning, magnetism and balance are included in *Supermotion*. Morrow will publish the books in the United States in spring 1983, price \$5.25.

Last, but very definitely not least, is **The Science Book** by Sara Stein, a book first published in



Darwin agogo on Tierra del Fuego, together with George Carter the *Beagle's* cabin-boy. (From *The Adventures of Charles Darwin*, reviewed here.)

them nice enough to dip into but not especially good as serious reference books. More on computers is to be found in the 40 double-page, large-format **Discovering Computers** which, with **Discovering the Weather**, is the latest in Longman's *Discovering* series (£6.95 each; Little, Brown, \$12.95 each). The writing is dull and the approach dry but the child already interested in either subject would be happy to be given them.

Far less routine and largely successful in their intent are numbers 9 to 12 in the series *How We Found Out About . . .* by Isaac Asimov, also from Longman (£2.75 each). Published in the 1970s in America by Walker, these four new volumes deal with **Energy, Germs, Antarctica and Dinosaurs** (what, no computers?), largely from a historical point of view. *Germs*, for example, starts with the first microscope and ends with the discovery of viruses. The usual childish angles are largely eschewed — there is almost nothing on the death of dinosaurs or solar energy — but a whole chapter on entropy. Not surprisingly, Asimov carries it off with ease.

Thorough assessment of the five books of experiments would have taken more time and children than were available. In

the United States and now available in Britain (Heinemann/Workman; £4.95, \$6.95). It is full of humour, zany facts (why don't cats beg for cookies? — because they have no taste receptors for sugar), and experiments to attract and educate children. To learn about kidney function, eat asparagus and follow your nose. Get to grips with the genome by cloning a plant (unfortunately for British readers, here the recommended species are all American) and with anatomy by checking out the chicken bones on a Sunday roast.

The Science Book is not simply a list of experiments. Rather it is an unorthodox primer, with unorthodox demonstrations, on much that is of interest to a growing child. There are sections on charges (electrical and magnetic), protists and plants. But the bulk of the book is concerned with physiology while chemistry and genetics get a reasonable look in. This is the only book of the whole batch to be centred on such sciences; for that and its refreshing approach, I give thanks and can forgive some errors of judgement. □

Peter Newmark, the father of a six-, a nine- and a twelve-year-old, is Deputy Editor of *Nature*.

Risibility research reviewed

William H. Press

WE TRY to keep it a secret, but we all know that research and teaching are not what science is all about. Rather those doubtless worthy activities are pale diversions from the real business of science, namely the invention, dissemination and elaboration of funny anecdotes about colleagues, parodies, doggerel and the occasional pithy aphorism or *bon mot*.

A Random Walk in Science, which was published about a decade ago as a collection of such fundamental material, has come to be held as a kind of *urtext* for all science, the ultimate peak of that pyramid which begins with primary publications at the bottom and then ascends to review articles, reviews of reviews and so on.

Now this sequel, collecting some 175 or so additional short pieces, can be added to that lofty edifice. Here, for everyone, there is something, whether whimsy like "The Use of Small Dogs in Physics Teaching" or broad jokes like the investigator who ate dehydrated food for 28 days and then gained 108 lbs in ten minutes while caught in a rainstorm. Parody ranges from the predictably amusing "I am the very model of . . .", to the arcane, such as Shelley's *Ozymandias* ("Two vast and trunkless legs of stone/Stand in the desert . . . Look on my works, ye Mighty, and despair! . . .") rewritten as the geology paper "Twin Limb-like Basalt Columns and Their Relationship to Plate Tectonics".

Scientists cannot resist applying scientific observation to their own endeavours, yielding, for example, a description of their university hierarchy, from the Dean who leaps tall buildings in a single bound, through Professor (leaps short buildings with a running start and favourable winds),

More Random Walks in Science. Compiled by Robert L. Weber. Pp.207. ISBN 0-85498-040-7. (The Institute of Physics/Heyden: 1982.) £9.95, \$19.50.

graduate student (runs into buildings) and finally department secretary (lifts buildings and walks under them). There is also a piece which rings true on the various types of obstructionists on committees and panels, and another on the "game" of refereeing. (The author's goal is to publish a worthless paper; the referee's is to have a major contribution to the field refused. "No matter what degree of rigor the author uses, the referee replies by saying that it is not the correct one.")

Anecdotes are nicely represented. We have the famous theoretician Sir Harold Jeffreys, consultant to an oil company, listening attentively to a full day's presentation and then offering his (only) advice: "I'm glad it's your problem and not mine". We have Bessel receiving from King Friedrich Wilhelm not the needed funds to improve his observatory, but rather a medal for having attained such excellent results with primitive equipment. We have Pauli in heaven, hearing with mounting impatience God's explanation of the proton-electron mass ratio and finally snapping back, "Wrong!". And Rutherford's memorable encounter in a glass-teapot works.

Literary hoax is represented by the paper coauthored by a cat (so that the sole human author could use the word "we" over an editor's objections), and by the biography of Claude Litre, the celebrated eighteenth-century maker of wine bottles after whom the volumetric unit is (putatively) named.

The volume also contains perhaps the finest scientific biography of Sir William Rowan Hamilton ever penned, here quoted in its entirety:

*Higgledy, piggledy
William R. Hamilton
Wrote on mechanics, in-
Frequently joked,
Wrote on quaternions,
Tackled intractable
Hydrodynamical
Problems, and croaked.*

While perhaps unsuitable as an introductory text, this volume will certainly fill the needs of advanced students and practitioners in the field. □

William H. Press is Professor of Astronomy and Physics, and Chairman of the Department of Astronomy, at Harvard University.

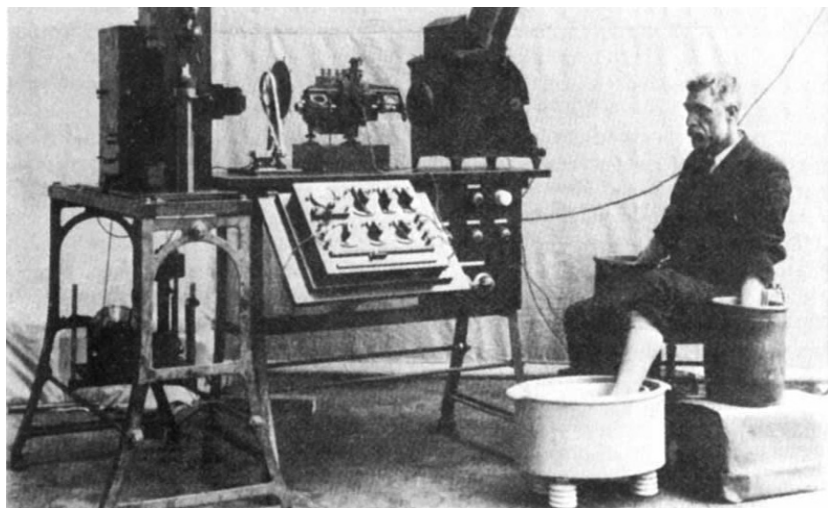
How to be a scientist

Walter Gratzer

Winning the Games Scientists Play. By Carl J. Sindermann. Pp.285. ISBN 0-306-41075-3. (Plenum: 1982.) \$15.95, £10.05.

WE ALL agree that there is more to science than doing research. The politics of professions in which scope for the exercise of power is limited or illusory, such as the academic or the ecclesiastical, tend to be conducted with particular ferocity and vindictiveness, redeemed only by the incompetence of most of the protagonists. It is a subject that invites satirical dissection: someone should do for our profession what A.P. Herbert did for (or to) the law.

I picked up Dr Sindermann's book with eager anticipation. The title is full of promise and from the dust-jacket the author, wearing a natty check and the suggestion of a knowing smile, looks at one appraisingly with gimlet eyes — clearly A Man that Waiters Spring to Serve. I averted my gaze and turned to page 1. But what was this? The first sentence averred that the book had been written for "thousands of serious dedicated scientists", such as myself, "to ensure that they [may] participate fully and joyously in a genuinely wonderful occupation". If this was a manual of gamesmanship for the



Courtesy Cambridge Medical Instruments Ltd.

From *More Random Walks in Science*.

Having fun in the laboratory? This doleful character appears to have doubts about the safety of the original electrocardiograph (c. 1911).

scientist it evidently owed little to Stephen Potter and all too much, if not to Lord Chesterfield then certainly to Dale Carnegie: how to win patrons and influence the Federation of American Biochemists.

There is, it is true, a sprinkling of anecdotes and many a genial aside, but there is not much levity, for Dr Sindermann's purpose is serious. His thesis is that for most scientists, the pleasures of the laboratory bench are in the long, or even the short run inadequate; and that if they are to enjoy a full and rewarding career they have no option but to throw themselves into the parasitic activities that beckon on all sides, such as attending and preferably organizing meetings, sitting on committees and running international commissions. He would evidently reject the proposition (first enunciated, I believe, by no less an authority than Professor Milton Friedman) that useless work drives out useful work. In short, while insisting that there is no substitute for prowess at research, Dr Sindermann urges his readers to join that travelling circus of Flying Dutchmen, whose chief and urgent preoccupation it is to keep as far away from their laboratories for as much of the year as possible, and leave the research in the calloused hands of their postdocs, technicians and PhD students.

As a guide to social and professional advancement in such company, the book is, so far as I can judge, excellent. Its most insistent message is that one must cultivate an uncompromisingly professional approach to all the activities in question — including even the successful pursuit of your quarry at the conference cocktail party. Dr Sindermann dispenses much good advice on how to give a paper at a meeting, how to make effective use of slides and how to deal with discussion. He descants on the best way to run a session and on the problems of organizing the whole affair yourself. Anxious novices will no longer have to discover the hard way where to go for financial sponsorship, how to cope with recalcitrant and temperamental foreigners and what to watch out for in drawing up an agreement with a commercial conference organizing company. Dr Sindermann writes here with an authority that can only stem from long experience and careful study.

On the more general aspects of the *arriviste's* upward trajectory, however, such as the craft of committee chairmanship (or in the Newspeak favoured by Dr Sindermann, chairpersonship), he has much less to say that is not a mite obvious. He does not offer such helpful hints in Advanced Chairmanship Technique as that once imparted to me by an expert in the field: ensure that the room is overheated and make large volumes of tea and coffee available, so that the ensuing discomfort will inhibit prolonged argument when eventually you introduce the issue of real importance. Again, it is related that

Maurice Bowra, a university politician of transcendent virtuosity, would overturn a ten to one majority against him by observing that an *impasse* seemed to have been reached, and perhaps therefore the meeting should be adjourned. Dr Sindermann's advice in his "chairman's creed" is more along the lines that a chairman must be firm but not domineering. Most of what he has to say might be more succinctly summed up in Senator Dirksen's well-known adage that the oil can is mightier than the sword.

In the later chapters there is a good deal of padding, and to my mind not much that is helpful when it comes to coping with the "midlife crisis" or with such alien forces as probing politicians from the outside world and women in the laboratory. It is curious that a book which encompasses such a wide range of opportunities and pitfalls — and even advises you how to deport yourself in the laboratory when in the throes of lust — makes not a mention of teaching. We are meant to conclude presumably that this is an occupation that plays no part in the process of getting ahead.

Dr Sindermann is an Establishment man. His advice is that you order your life according to Establishment rules, and then you too may become known for being noted. He has no patience with the unsuccessful and mediocre, who, he holds, are simply not trying: you should show them no mercy. His book may well help to reclaim some of these fallen, and should give encouragement to the walking wounded in the battle for academic survival. But where it may comfort the afflicted, it will certainly not afflict the comfortable. There are, it seems to me, more useful maxims for young scientists, struggling to prevail in a hostile climate, than any that Dr Sindermann has to offer. One that seems to me to encapsulate an enduring truth comes from Alexander Cohen's *Journal of Irreproducible Results* and goes like this: if a research project is not worth doing at all, it is not worth doing properly. Cap that, Dr Sindermann. □

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Now you solve it, now you don't . . .

David Jones

Aha! Gotcha: Paradoxes to Puzzle and Delight. By Martin Gardner. Pp.164. Hbk ISBN 0-7167-1414-0; pbk ISBN 0-7167-1361-0. (W.H. Freeman: 1982.) Hbk \$15.95, £11.20; pbk \$8.95, £5.60.

MARTIN Gardner is probably best known for his feature in *Scientific American*, "Mathematical Games", which ran regularly from 1956 to 1981; but he has also published many other articles and books, both light-hearted and serious, on mathematical topics. *Aha! Gotcha* is definitely light-hearted, but still true to its subject, and similar in format and spirit to its companion-volume *Aha! Insight*.

Both books contain about 70 self-contained entries, each exploring some intriguing mathematical trick, puzzle or theorem. *Aha! Gotcha* deals with paradoxes, pointing out the role they play in sharpening mathematical reasoning, and presenting them in six sub-divisions: logic, number, geometry, probability, statistics and time (this last strays over the boundary of mathematics into physics).

Here are discussed the familiar paradoxes of self-referring statements (the lying Cretan), infinite sums and curves (the Snowflake), probabilistic fallacies (Pascal's wager) and the Zeno paradoxes (Achilles and the tortoise). Here also are many more modern and subtle paradoxes: the Unexpected Tiger, Newcomb's paradox of the perfect thought-reader, the voting paradoxes which subvert our hopes for a perfect democracy.

Each entry provides a simple but well-reasoned discussion of the chosen paradox, and some examples — one of them in strip-cartoon format. These cartoons are characteristic of the *Aha!* books; they are highly stylized drawings by Jim Glen, and feature characters such as Dr Zeta, the extraterrestrial scientist, and Mr Randi, the world-famous magician. I can imagine them either delighting or infuriating the reader. Intellectually, they provide a sort of pictorial commentary on the text which makes its reasoning more accessible, and gives the book a cheerful and whimsical atmosphere.

Aha! Gotcha should appeal to students and non-specialist readers who would be discouraged by a more formal presentation; at the same time even professional mathematicians may be glad to see their subject sketched in this ingenious way. Many of the paradoxes are not yet resolved — does the occurrence of a purple cow really provide support for the assertion that all crows are black? For those who wish to pursue such questions further, the book has a useful reference-list of further reading. Less dedicated readers will merely enjoy puzzling over the paradoxes and springing them on their friends. □

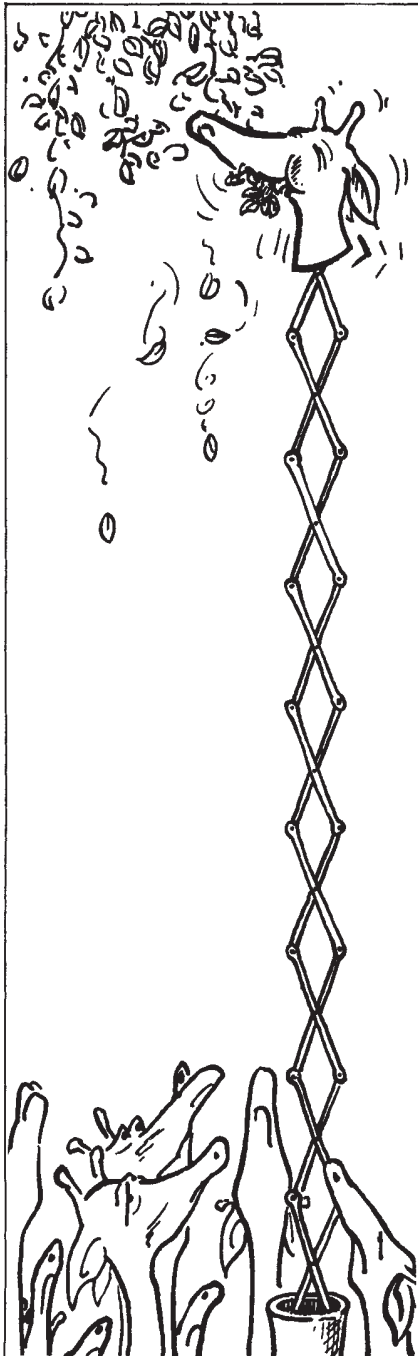
David Jones is a Guest Member of Staff with the Physical Chemistry Department of the University of Newcastle upon Tyne. His book, *The Inventions of Daedalus*, derived from his weekly column in *New Scientist*, has just been published by W.H. Freeman.

Darwin and evolution: the comic cut

J.S. Jones

Darwin for Beginners. By Jonathan Miller and Borin van Loon. Pp.176. Hbk ISBN 0-906495-95-4; pbk ISBN 0-906495-96-2. (Writers and Readers/W.W. Norton/Pantheon: 1982.) Hbk £7.95, \$11.95; pbk £2.95, \$3.95.

EINSTEIN, Marx, food, Freud, capitalism — and Darwin. These are some of the titles in a series of illustrated books "for beginners" which discuss complex subjects in a simple way using a mixture of cartoons



Stretching a point about evolutionary theory. One of Borin van Loon's illustrations in *Darwin for Beginners*.

and text. How does Charles Darwin emerge from his treatment as the thinking man's Andy Capp?

The short answer is: remarkably well. Jonathan Miller gives us a concise and clear account of Darwinism, which is complemented by some two hundred crude but powerful illustrations by Borin van Loon. Darwin's predecessors, his life and the *Origin of Species* are covered in a body of text enough to provide only the preface for most books on evolution. We learn not only of the well-known elements of our hero's life, but also of neglected episodes such as Captain Fitzroy's early distrust of Darwin because the shape of his nose betrayed signs of laziness and hesitancy. The laziness — which provoked his father to exclaim that "You care for nothing but shooting, dogs and rat-catching. You will be a disgrace to yourself and all your family" — disappeared early, but the hesitancy remained for the rest of his days.

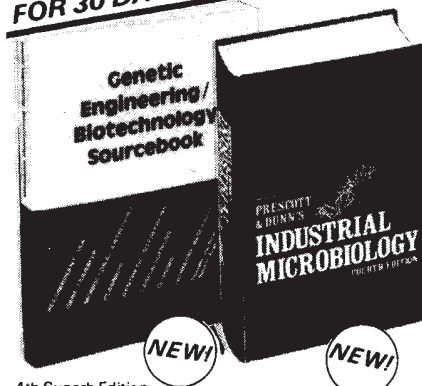
Darwin's lack of confidence and his hypochondria are often blamed on the effects of Chagas' disease (which he may have contracted in South America). Here a dramatic illustration shows him cowering before an infected mosquito — which is unfortunate as the vector is a wingless blood-sucking bug. There are many quotations from Darwin's writings, published and unpublished, including his musings on the advantages of a "nice soft wife on a sofa"; advantages which include, according to the accompanying cartoon, sharing a bed with Freud and Marx.

The treatment of Darwin's predecessors and his own work is outstandingly clear. We hear of Cuvier, to whom evolution was a process of stability interrupted by miraculous change, and of Paley, who saw in the perfection of living things the proof of an intelligent designer. That of neo-Darwinism and the "modern synthesis" is less successful, and shows signs of confusion. There is the compulsory nod towards neo-Cuvierism — or evolution by jerks as it is known to its critics — in which mysterious forces lead to evolutionary equilibria punctuated by rapid change, but nothing on neo-Paleyism, the view that all animal structure and behaviour is of necessity perfectly adaptive and is hence always a proof of strong natural selection.

Nevertheless, the book as a whole is a success. It can be recommended to biologists who have ceased to be entertained by evolution as well as to newcomers to the subject. Darwin, Doonesbury, Dennis the Menace: in this post-literate society to be immortalized in a comic strip is the ultimate accolade payable to a great mind. What next — a cartoon version of *Principia Mathematica*? ☐

J.S. Jones is in the Department of Genetics and Biometry at University College London.

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Knocking about in the nineteen-eighties

Bernard Dixon

The Cult of the Expert. By Brian J. Ford. Pp.194. ISBN 0-241-10476-9. (Hamish Hamilton: 1982.) £5.95.

ONE of Dwight D. Eisenhower's more memorable remarks was that "public policy could itself become the captive of a scientific-technological elite". That forecast was underscored some years later by Dr Herbert York, Defense Department adviser to both Eisenhower and Kennedy, when he warned that the possible decision to initiate nuclear annihilation was passing "from the hands of statesmen to lower-level officers and ultimately computing machines and the technicians who program them".

Clearly, this is a theme, with parallels in fields as disparate as health and nutrition, communications and education, which is worthy of serious attention. Yet it has not been widely discussed — certainly not in books. Theodore Roszak's *The Making of a Counter Culture* (Faber & Faber, 1970) touched on the problem, but was more concerned with the domination of our society by the products and ideas of science than with the role of experts *per se*. So there certainly is an opportunity for a keen examination of specialists, their habits and attitudes, and their effect on the rest of us — citizens, consumers, customers, captives.

The Cult of the Expert appears at first sight to be such a book. It is (to take his conclusion first) Mr Ford's fleshing out of the following thesis:

From the way you are born to the manner of your dying, great and earth-shattering decisions are taken behind closed doors by people who may well have closed minds. For the Expert all that matters is that they are seen to have a monopoly on power. Their long and meaningless words, elaborate and expensive apparatus stacked in high security buildings, the machines that they secretly know no more than anyone else how to use for the good of the people, are all part of today's power structure. The omnipotence of the Expert pronouncement and the unchallengeable right to spend huge sums of money gratifying a lust that evolved only to be gratified; these are the pattern for the future.

In support of these somewhat inelegant propositions, Mr Ford tells us at great length that experts employ contorted language. He provides many examples such as "mobility transportation system" for "car", quotes a passage we may have missed from *Private Eye* showing that the word "situation" has become comic, and uses a menu to compare "plain language" with French. He states that Ulcerative Dermal Necrosis ("as far as it means anything at all") really means that ulcers of dead skin form on the surface of fish, and he devotes an entire chapter to rules such as "never use one word where many will do".

Mr Ford recounts the banning of cyclamates, the N-ray story, the polywater affair, the Kammerer and Burt episodes, and the work of Stanley Milgram, to show science in a variety of bad lights. He says that an Expert could ban air, and explains that nitrogen and its other constituents can be dangerous. He criticizes new maths and decimalization (predicting that Experts will next want to divide the year into 100 parts). He then complains about bank cards and gives many examples of "the way in which the infectious enthusiasm for believing in computers has come close to harming our lives", before vouchsafing that there are innumerable other such examples. He gives pupils rules such as "Do just what the teacher says, and on no account argue" and opines that the purpose of a university is "to provide a closetted and confined period of indoctrination for malleable minds". Finally, with embarrassing innocence of the real world, Mr Ford tells us that "elaborate and expensive research institutes" are needed only for Experts to perpetuate themselves, and that libraries will go on paying inflated prices for journals.

Anyone setting out to survey such (some

may think familiar) territory should decide whether their book is to be humorous or serious, and whether the aim is to flatter readers or make them feel uneasy. Mr Ford has done neither. He begins by taking a swipe at his readers' poor education and calling them "ordinary people", but then expects them to join in his sarcastic superiority. He enjoys his own knockabout fun but sours it all by being bitchy. He gets serious things wrong (the butter-margarine controversy was about the contribution of their respective fatty acids to coronary heart disease, not about which was more fattening) while trying to be witty by assuring us that Experts are above the law. And his publishers do not help by producing what they (presumably) intended to be a rib-tickling book with a dust jacket highlighting the author's "acid pen". Curious.

Mr Ford is not, I understand, a qualified scientist. So his words come with considerably less clout than those of, say, Sir Peter Medawar when he gently mocks some of his fellow experts. But then the very idea of Medawar writing *this* book is much funnier than anything Mr Ford has to say. □

Bernard Dixon, formerly Editor of *New Scientist* and *European Editor of Omni*, is a science writer and consultant.

Pictures, prose and a world of vegetation

Philip Stott

Green Planet: The Story of Plant Life on Earth. Edited by David M. Moore. Pp.288. ISBN 0-521-24610-5. (Cambridge University Press: 1982.) £12.50, \$27.50.

THE many recent attempts of science publishers to blend the visually attractive with the academically acceptable have not always proved felicitous. Solid content has all too often yielded to the simply picturesque. One notable exception is Duvigneaud's splendidly gallic *La Synthèse Écologique* (Doin, Paris; 1974). This new encyclopaedia of plant ecology and geography is another.

There are two clear reasons for the book's success. First, the text, which has been skillfully compiled from the separate contributions of 30 specialists, dominates the book and is, in the main, both comprehensive and accurate. Secondly, the visual material is used to illustrate and exemplify the text rather than, as is so often the case, the words being mere adjuncts to pretty pictures. In addition, it is especially gratifying to welcome a general work on biogeography which acknowledges nearly all of the main traditions of the subject. In the seven well-constructed chapters, the encyclopaedia thus embraces the key aspects of ecology, of plant geography in

its classical sense — meaning the study of the spatial distribution of plant taxa over the surface of the Earth — and of the role of man in nature. This last theme is perhaps the least satisfactorily covered and I was sorry to see no mention of the important applied textbooks of I.G. Simmons in the bibliography at the end of the book.

Obviously, in a survey of such breadth, there will be some blemishes. The term "ecology", for example, was not coined by the German biologist, Ernst Haeckel, in 1869 (p.21), but appears as early as 1858 in a letter of the American essayist and nature-lover, Henry David Thoreau; moreover, Haeckel himself first used the term in 1866. It is pleasing, however, to see both autecology and synecology adequately discussed.

Cladistics is well explained, as one would expect, by Heywood in his precise and useful entry on taxonomy and systematics, but nowhere is the possible phytogeographical significance of cladistic relationships explored. The closely related topic of vicariance biogeography is largely ignored, save for a short passage on vicariance written in a more traditional vein. It is also regrettable that many of the plant and vegetation distributions are still mapped on non-equal-area projections, which

unacceptably exaggerate the northern latitudes, whilst the large and well-chosen glossary is occasionally somewhat opaque in its definitions.

But these are all quibbles. This encyclopaedia is both elegant and informative. The editor and his contributors are to be congratulated on providing what is really a colourful and modern Polunin (*Introduction to Plant Geography and Some Related*

Sciences, published by Longman in 1960). It should prove of enormous value to both sixth formers and undergraduates, as well as to a wider readership of naturalists and botanists. □

Philip Stott is a Lecturer in Biogeography at the School of Oriental and African Studies, University of London, and author of Historical Plant Geography (George Allen & Unwin, 1981).

as the first report of the giant panda.

In the late eighteenth and early nineteenth century, Sir Joseph Banks, First as a collector, later as the impresario of other men's travels, commanded the field. His patronage, allied with that of the Royal and Horticultural Societies and Kew Gardens — all three institutions firmly under Banks's wing — sent professional collectors across the globe; South Africa, Australia, China and Japan were all areas in which he was interested. Later other patrons or nurserymen financed major expeditions, a pattern that lasted to relatively recent times and the journeys of Ludlow and Sherriff and of Kingdon-Ward. Now the flood of new plants has slackened a little and efforts are being made to prevent the loss of older species driven into neglect by the vagaries of horticultural fashion.

The subject of Mr Fisher's book must appeal to any gardener with the slightest interest in the history of the plants he or she grows. The text, however, is not always



from *The Illustration of Plants & Gardens 1500-1850*

accurate; nor are the captions to the illustrations. No one reading the book would guess that the Chelsea Physic Garden still survives, although its future seems once again to be in jeopardy. And it is said that Robert Brown, his Australian travels over, was "afterwards librarian of the Linnean Society". He did not stay in that position, though, for he was Banks's botanical heir, an eminent scientist in his own right and eventually President of the Linnean too.

The author notes of John Bartram, an eighteenth-century Quaker naturalist living in Pennsylvania, "His spelling remained consistently unreliable". A similar warning is needed here, for Mr Fisher's book appears to have escaped both copy-editing and proof-reading. Spelling in English and Latin is equally eccentric: even dates are often wrong. Let the buyer enjoy this pleasant book, but also beware of trusting the accuracy of the details. □

Sandra Raphael is Senior Editor (Natural History) in the Dictionary Department of Oxford University Press. She is currently working on a new edition of Wilfrid Blunt's The Art of Botanical Illustration.

Other men's flowers, other men's gardens

Sandra Raphael

The Illustration of Plants & Gardens 1500-1850. By Vera Kaden. Pp.106. Hbk ISBN 0-11-290375-4; pbk ISBN 0-11-290331-2. (HMSO/Kraus Thomson: 1982.) Hbk £12, \$25; pbk £6.95, \$14. *The Origins of Garden Plants.* By John Fisher. Pp.338. ISBN 0-09-463760-1. (Constable: 1982.) £12.95.

VERA Kaden's collection of pictures taken from botanical and gardening books in the library of the Victoria and Albert Museum made a good first impression on me by using a beautiful Ehret drawing of a lily — one of my favourites — to adorn its dust jacket. The source of the illustrations selected indicates that the books and manuscripts were chosen for aesthetic appeal rather than purely botanical interest, but such a limitation does not, of course, exclude most of the greatest plant books; nor, indeed, does it detract from the book's contents.

A short introduction, sparingly decorated with black-and-white illustrations, canters briskly through a potted history of garden design, with excursions to provide necessary details of herbals and flower books and the processes by which they were illustrated. There is also a brief account of floral symbolism.

The remaining two-thirds of the book is made up of plates, about half of them in



from *The Illustration of Plants & Gardens 1500-1850*

colour, all with descriptive captions. The arrangement is more or less chronological, starting with the earliest woodcuts of plants

from the late fifteenth century and ending with a couple of plates from Victorian albums of flower paintings. The plants and the gardens go hand in hand, with pictures to show changing tastes in design, from the enclosed mediaeval garden to architectural Italian ones, formal patterns from France and the English style of landscaping. Nearly all the colour plates, reasonably enough, are devoted to the work of the flower painters, with Maria Sibylla Merian, Ehret, Redouté and Franz Bauer representing the greatest of them. Altogether the book makes an attractive guide for those taking their first steps in garden history.

John Fisher's book, *The Origins of Garden Plants*, also offers background information to the curious gardener, in this case on the subject of plant-hunting and the introduction of some of the botanical immigrants now comfortably settled in the British Isles. The book starts with a couple of chapters on the native plants of Britain before going on to those cultivated for medicinal and other purposes in mediaeval times. Then we are launched into the search for decorative plants, which very often ran parallel with the exploration of new colonies. Almost every part of the world has been visited by amateur or professional plant-hunters, with obvious consequences in the range of plants now available to us — the rhododendron belt of the Home Counties would not have been possible without Hooker's Indian journeys and the Chinese collections of several French and British explorers; and several of the species of conifer covering all too many of our hills are the descendants of the North American discoveries of David Douglas and his contemporaries.

Collectors in Europe began with attempts to identify the plants described by classical writers. The seventeenth century brought the first great wave of introductions from the New World, many (including *Tradescantia virginiana*) via the gardens of the Tradescants, father and son. The French in Canada and the southern part of the continent sent back plants to Paris, the Spaniards in South America to Madrid. French missionary priests, particularly in China, sent home plants as well

Bees for science and bees for keeping

John Powell

Bees and Mankind. By John B. Free. Pp.155. ISBN 0-04-638001-9. (George Allen & Unwin: 1982.) £9.50, \$17.95.

DR JOHN Free is internationally acknowledged as an authority on bees and pollination; his published work in the scientific literature is extensive. Any book from such a source is therefore bound to be looked upon as of some importance in beekeeping circles.

It is interesting to note that of Dr Free's books to date, half have been written for children and it is evident that he has mastered the art of expressing a complex subject in a straightforward and lucid manner. In confirmation of this, *Bees and Mankind* is a fascinating and easily readable work, though its structure is unusual. The book is split into three parts entitled "Bees: Solitary and Social", "Honeybees" and "Beekeeping: Past and Present". The reader, however, is more likely to regard the text as divided into two.

Parts I and II together provide a brief account of two selected solitary bees, *Osmia rufa* and *Megachile rotundata*, and a more detailed treatment of bumblebees and honeybees. Each chapter is broken into short sections, each headed by a quotation from such diverse sources as

Virgil and J.G. Whittier. It is the sharp contrast between the cool text of Dr Free and the colour of some of the poetry that provides much of the charm of this book, though I found it took some time to get used to it. This then is not a book to dip into — at least not at first acquaintance.

There is a distinct change of style when we enter Part III, effectively the second half of the book, and move from the strictly factual material to a narrative of man's relationship with bees over 9,000 years, from primitive honey-hunting to the sophisticated mechanization of the late twentieth century. This must be one of the most complete accounts available and John Free's wide experience of the bee species involved is evident in the way he has been able to make a coherent whole from the often fragmentary evidence. There is, too, more than an interesting story here; in their unobtrusive way bees have had a profound influence on man's social, religious and cultural development.

Like the bees themselves, the sting of the book comes in the tail. The closing pages deal with the influence and availability of pollinating insects on agricultural production, both in intensively farmed areas and in developing countries.

The book is liberally illustrated and is beautifully set-out and produced. Although the subject matter is vast there is throughout an element of speculation which binds the whole together, be it the socialization of bees or man's manipulation of that social organization. It would be a pity if *Bees and Mankind* remained solely within beekeeping circles; it could be read profitably by all who are involved in conservation and planning, agricultural development, international aid programmes and anthropology. □

John Powell, a research physicist, is author of *The World of a Beehive* (Faber & Faber, 1979).

High in Africa

Margaret Sharman

Kilimanjaro. By John Reader. Pp.84. ISBN 0-241-10683-4. (Elm Tree, London/Universe: 1982.) £12.95, \$25.

KILIMANJARO is not only Africa's highest mountain, and so a magnet for climbers, it is also an ecologist's delight. Within a few miles one finds tropical forest, alpine flora, near-desert, glaciation and the world's biggest volcano. Such variety provides scope for the excellent photographs, ranging from majestic landscapes to haunting close-ups, with which John

Reader adorns his record of one man's pilgrimage to Kilimanjaro.

One usually sees the mountain for the first time as a backdrop for the migrating herds of the Serengeti Plain, the elephants of Tsavo, or even, on a clear day, the ex-colonial suburbs of Nairobi, over 100 miles away. It rises from the burning heat of the thorn savannah, and it is no wonder that the reports of missionaries Krapf and Rebmann in the mid-nineteenth century were rejected as the imaginings of fevered brains and poor eyesight.

Yet tales of snow-capped mountains in Africa were not new. As an introduction to the highly personal account of his first climb, the author traces the story through the centuries, as Europeans overcame the obstacles — adverse geographical conditions, slave traders and the spears of tribesmen defending their homeland against white men bent on "discovering" Africa. (I am dismayed, incidentally, to find that the kink in the Kenya-Tanzania border is not the result of Queen Victoria giving Kaiser Bill the present to end all presents.) African legends, too, there are in plenty; and tales of those who were intrepid enough to brave the "fiery beings" and the fingers that "died", to obtain samples of "the silver-like stuff [which] when brought down in bottles, proved to be nothing but water".

Climbing the mountain is one of the things Westerners who live in East Africa aim to do "one day". It is everybody's mountain in that, while it is climbable without expertise, the twin peaks of Kibo and Mawenzi provide severe challenges, some of which are here described with concomitant gruesome tales of misadventure.

The snows of Kilimanjaro are melting rapidly (the ice has been in retreat since c.1700), and the melting process is responsible for the fantastic ice edifices which are among the most striking images in the book. Lower down in tropical splendour are the giants — the lobelias, and the groundsels which grow up to four metres high and may be 100 years old. How plants withstand temperature extremes and at the same time cope with intense ultraviolet radiation and the threat of being exhumed by solifluction is explained in the last section of the book.

Near the mountain's summit are found cryptozoic spiders, and crystallized butterflies which have been blown up by the "anti-trade wind" — a wind which explains the conventional picture of a snow-white summit riding high on a cloud layer and apparently completely detached from the earth below. This is the one photograph which every tourist takes home, even if only on a postcard, and which John Reader omits. But with 82 colour plates one cannot cavil at that. Here is an attractive and very readable book.

Margaret Sharman is a freelance editor specializing in books on African history.

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Half and half in the national parks

Brian Bertram

A Field Guide to the National Parks of East Africa. By John G. Williams and Norman Arlott. Pp.336. ISBN 0-00-219215-2. (Collins/Stephen Greene Press: 1982.) £7.95, \$29.95.

LIKE ALL Gaul, this handy field guide is divided into three parts. The first part surveys the national parks, game reserves and other faunal areas of Kenya, Tanzania and Uganda, the second is a guide to the larger mammals and the third a guide to the commoner birds of East Africa.

Unlike all Gaul, the standard of the three parts varies widely. The 70 pages on the mammals are well-presented and accurate; they are in effect the same as in the original 1967 version of this field guide. With the help of 13 pages of illustrations, they enable the visitor to identify almost any mammal he is likely to encounter. The 92 pages on the commoner birds are also good, and are the completely new part of the book. They replace the section in the previous edition which dealt with the rarer birds, and instead provide a condensed version of its companion volume on the birds one is more likely to see. Fifteen pages of effective colour illustrations help in the identification.

But as for the remaining part, "Oh dear" was my reaction. The opening 160 pages on the national parks and other wildlife areas are absolutely riddled with mistakes, and are going to mislead a great many people. Partly this is because they are hopelessly out of date, and have not been changed from the previous edition. Thus, to pick out just a couple of examples, the area of Meru National Park is now less than half the area John Williams states, and the "motor boat available for anyone wishing to explore the country from the rivers" was actually burnt to ashes at least a dozen years ago.

Other errors abound on the maps, caused partly by some careless re-drawing of long out-of-date maps. Thus roads (in Tsavo) and boundaries (in Amboseli) are

often in the wrong place, and lodges are omitted (in Serengeti) or misplaced (in Tsavo). Even whole reserves are misplaced — one is shown over 200 km away from where it actually is. Advice on routes is often wrong and details in the species lists for the parks are faulty; for example the abundant hippo is omitted from Tsavo (despite a successful film about them there), and Patas monkeys have not been seen in Meru by anyone who has worked there for long. And so on.

This is very sad, because there is a wealth of knowledgeable people in East Africa who could have weeded out almost all of these mistakes, and made the book far more useful. As it is, one has to say "Don't trust the first half of it". But there is no other comparable guide, so if you are going to East Africa you had better get hold of it, warts and all. And take with it a pinch of salt and some proper maps.

Brian Bertram is Curator of Mammals at The Zoological Society of London.

Private lives

John R. Krebs

The Discovery of Animal Behaviour. By John Sparks. Pp.288. ISBN 0-00-219061-3. (Collins/Little, Brown: 1982.) £12.95, \$24.95.

FOLLOWING along the now well-used trail opened up by *Life on Earth*, *The Discovery of Animal Behaviour* is the book of the television series recently shown on BBC2. The text follows almost verbatim the script of the programmes, and although well written it loses some of its impact when not accompanied by the film. But pictures there are in plenty; every second page is a double-sided colour plate of high quality.

For those unfamiliar with the series, the book is essentially a history of ethology, highlighting the contributions and circumstances of key figures from the middle ages to the 1960s. The "stars" include Ray, White, Leroy, Darwin, Romanes, Spalding, Loeb, Watson, Skinner, Pavlov, Lorenz, Tinbergen and von Frisch, a fairly uncontroversial list. In spite of its title, the last two chapters of the book (covering the mid-twentieth century) are almost exclusively about ethology and sociobiology: psychologists faded out after Skinner and Harlow.

Those hoping to gasp at arcane marvels of animal behaviour or to be enlightened about the big issues in present-day ethological research may be disappointed, for this is as much an account of the people as of their subject. It is hardly gripping stuff, especially for those not familiar with animal behaviour; after all, most naturalists are men of quotidian habits.

One of the nice features of the book is the way in which the discoveries and attitudes of individuals are related to their personal circumstances. For many the stimulus and opportunity to study animal behaviour was linked with relaxing country residences — a mill by an Austrian lake, a rambling house close to the Danube, a country cottage at Dunskaith; in stark contrast, we are told that the theoretician W.D. Hamilton did much of his thinking while waiting at Waterloo Station!

Another recurrent theme is how easily scientists were led to generalize their findings to an unreasonable point. Loeb, the discoverer of phototaxis considered whether the human mind could be explained in terms of taxes and tropisms; Romanes asked whether spiders might appreciate music; and Schelderup-Ebbe, the man who discovered dominance hierarchies, subsumed the relationship between God and the Devil in his account of social organization. It is easy to smile, but less easy to see whether or not one has one's own musical spiders. □

John R. Krebs is Lecturer in Zoology at the Edward Grey Institute and E.P. Abraham Fellow of Pembroke College, University of Oxford.

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REASONS

Home on the range — wildebeest (gnu) in the Amboseli National Park, Kenya. The snow-capped Kilimanjaro rises in the background. (Photograph David Keith Jones.)

And the gentlemen went there two by two

Bernard Stonehouse

Voyage Through the Antarctic. By Richard Adams and Ronald Lockley. Pp.160. UK ISBN 0-7139-1396-7; US ISBN 0-394-52858-1. (Allen Lane/Knopf: 1982.) £8.95, \$13.95. *Antarctic Wildlife.* By Eric Hosking and Bryan Sage. Pp.160. ISBN 0-7099-1215-3. (Croom Helm/Facts on File: 1982.) £12.95, \$22.95.

ANTARCTICA and the Southern Ocean are among the few remaining bastions of wildlife, though with a long history of depredation at the hands of human beings. Southern fur seals were hunted almost to extinction on the peripheral islands in the eighteenth and nineteenth centuries, and whale stocks devastated in the present century. Elephant seals and even penguins were boiled by the thousand for the golden oil in their blubber. Now the killing of the larger animals has almost stopped; attention turns to harvesting the fish and krill that will form man's final bonanza from the Antarctic.

For many years scientists and their support teams had sole rights of travel in the far south. Today tour ships are regular visitors, loaded with enthusiastic passengers who saw Alaska last year, the Galapagos the year before, and are now doing Antarctica. Tourists on the hallowed ground where Scott, Amundsen and Byrd once stood? Yes, and good luck to them. Nowhere in the world is the scenery grander and less spoiled by human artefacts; nowhere do seals and seabirds gather in more spectacular concentrations; and nowhere are wild animals more readily approachable by nature-lovers with camera, notebook and wide-eyed wonder. The two books reviewed here arose from tours laid on by Lindblad Travel, one of the earliest operators to brave the fury of stormy seas, elitist scientists and anxious administrators to take ordinary people (well, ordinary *wealthy* people) to the Antarctic. As a result, thousands have seen the southern wonderland who would otherwise have been excluded, and there are correspondingly more who can write about it, read about it and, if necessary, root for it from first-hand knowledge.

Voyage Through the Antarctic, the smaller and cheaper volume, is the narrative of a journey from Tierra del Fuego to Stewart Island, New Zealand, via the South Shetland Islands, Antarctic Peninsula, Peter I Øy and the edge of the

Pacific sector pack ice, calling at McMurdo Sound, skirting Cape Adare and the Balleny Islands, and visiting Macquarie and Campbell Islands on the homeward run. Two generations ago this voyage would have been regarded as hazardous in the extreme; even now it is a voyage that polar scientists (myself among them) would be glad to experience for the research opportunities offered. Yet this and other trips like it are the stock-in-trade of tourist operators these days. Richard Adams, naturalist, novelist (*Watership Down*, *Shardik*, *The Plague Dogs*) and RSPCA luminary tells the plain tale, which naturalist and writer Ronald Lockley (*Man against Nature*, *Orielton*, *Ocean Wanderers*) embellishes with italicized passages of concentrated natural history.

This may well have been a practical way (perhaps the only way) of combining the talents of two celebrated writers who live far apart and write in widely-diverse idioms. For me the device fails; the two voices sing in different keys. Both authors are self-conscious, Adams, the jolly tourist, Lockley, lyrical-authoritative; both have written far better, and neither rises fully to what could and should have been a splendid occasion. Peter Hirst-Smith's photographs too are disappointing. The few pages of colour glow excellently, but

the many black-and-white prints spread through the text could have been selected more rigorously and printed less muddily; the best are fair, and the worst have a potato-print quality that does them no justice. But this is a good adventure story, with a lot of information and a deal of enthusiasm for Antarctica and its animals that make the book worthwhile.

Antarctic Wildlife is for me a much more satisfactory book, larger and almost fifty per cent dearer, but a far better production all round. This is not the story of a voyage; it is an account — indeed a celebration — of Antarctic life, seen through the perceptive eye of Eric Hosking and discussed in scholarly but readable vein by Bryan Sage. The Old Master has lost none of his touch; these are superb photographs, printed to a high standard in both colour and black-and-white. I cavil only at a designer who on occasion lets the central gutter spoil the layouts unnecessarily. The seven chapters cover the historical background, continental, maritime and subantarctic ecology, and the life histories of penguins, albatrosses, other birds and seals; there is a final chapter on wildlife photography that many will find valuable. *Antarctic Wildlife* is an authoritative and beautiful volume, a book fit to match its subject. □

Bernard Stonehouse is at the Scott Polar Research Institute, University of Cambridge, and editor of *Polar Record*.

The natural history of mammals anew

I.J. Linn

Mammals in the British Isles. By L. Harrison Matthews. Pp.208. ISBN 0-00-219738-3. (Collins: 1982.) £10.95.

A NEW title from the prolific pen of Dr Harrison Matthews is always a significant event in the world of theriology; and this is a new title, not merely a new edition of his earlier book, *British Mammals*, in the same *New Naturalist* series.

As both author and publisher are at pains to point out, the tremendous flowering of theriological literature which has taken place in the past 20 years or so has enabled the author to dispense with much of the basic introductory material which was necessary in the older book, and to follow through a specific line of thought with simplicity and clarity. That line is the one which amateur naturalist and professional zoologist alike contemplate with wonder and delight, namely the extraordinary detail and perfection of the adaptations of animals, in this case British mammals, to the environments in which they live. It is the line of thought which has given us the academic disciplines of sociobiology and behavioural ecology, and

it also generates many of the questions which thinking people of many differing ages and backgrounds ask of biologists — questions which are often very difficult to answer. This book will help all of these people.

Dr Matthews lays a firm foundation by spending most of the first third of the book reviewing the geological, climatic and human influences which have shaped the range of habitats available to British mammals. He then goes on to write lucidly and effectively about the various facets of ecology and behaviour which define a mammal's adaptivity, presenting hard scientific information and modern theory in a palatable and easily absorbed manner.

The book as a whole is spiced by Dr Matthews's trenchant and perceptive comments upon humanity, past and present. It is an excellent introduction to the natural history of British mammals for anyone, amateur or professional, who is interested in real animals in the real world.

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Poles apart

Wildlife in the Arctic, specifically the polar bear, is the subject of *Lords of the Arctic* by Richard C. Davids with photographs by Dan Guravich. The book is an account of scientific observations, conservation problems and the authors' experiences in their search for this elusive animal. Published by Macmillan, New York, *Lords of the Arctic* costs \$29.95.

Birds from the kitchen window . . . and much further afield

John Andrews

THE KITCHEN window is the starting point for many people's interest in birds — an interest which they may never take beyond the front gate. **The Garden Bird Book**, edited by David Glue (Macmillan, London, £7.95), is right up their street. Compiled by the British Trust for Ornithology, its aim is to help people encourage birds into their lives by providing suitable food and shelter. It contains lots of practical advice applicable to the small suburban garden as much as to the fortunate minority whose spacious grounds attract woodpeckers and water rails. More than that, the text is thoroughly interesting, and packed with insights into the lives and survival strategies of common birds. Well-illustrated, this is certainly a book to enthuse the garden bird-lover and perhaps encourage forays further afield.

Anyone who does venture the transition from bird-lover into bird-watcher must soon attempt to choose from a plethora of field identification guides. Little has been added to the current choice by John Gooders and Terence Lambert's **British Birds** (Collins/Stephen Greene Press; £12.95, \$39.95). On the one hand, Lambert's illustrations though aesthetically pleasing are of little help to field identification: indeed, as printed, some of the colour tones are positively misleading. On the other hand, John Gooders' text is largely field-identification material inappropriate in a book which might fit the parcel shelf but certainly not an anorak pocket. In style and format, the book is very reminiscent of the Readers Digest/AA *Book of British Birds* published in 1969 which remains the more readable and entertaining volume.

Field identification skills usually depend less on feather-perfect knowledge of plumage than on knowing where and how different species comport themselves — just as most people recognize acquaintances at a distance without any ability to describe them in detail. For the fairly inexpert, **Birdwatching on Inland Fresh Waters** by Malcolm Ogilvie (Severn House, £8.95) provides this kind of helpful information on identification and basic ecology for a group of habitats rich in birds and accordingly popular with birders. Opening with a description of Britain's freshwater habitats, the author, who is on the Wildfowl Trust staff, moves on to discuss many aspects of wetland bird adaptation and behaviour, and concludes by explaining some of the current research and conservation work directed at these birds.

Strangely, many bird-watchers seem happy to go through life without ever understanding how the objects of their identification skills actually function. For those who are moved to do so, a good and not overly technical account is provided in

the book **How Birds Work** by Ron Freethy (Blandford/Sterling; £8.95, \$15.95). The author has evidently reviewed the current literature to produce an up-to-date explanation of opinion on evolution, structure, flight, respiration, feeding, the nervous system, breeding, migration, behaviour and distribution. Unfortunately, a rag-bag of illustrations adds nothing to the book's appearance and little to its information.

One area of common ground between bird-lover, bird-watcher and the scientific ornithologist (pompous fellow!) is the almost universal appeal of birds of prey. In



Study of a heron, by Eric Ennion. The illustration is taken from *The Living Birds of Eric Ennion*, a collection of drawings and paintings which displays the late Dr Ennion's remarkable and varied talents as writer, ornithologist and artist. The book includes a commentary by John Busby and is published by Gollancz, price £9.95.

Britain, a peregrine falcon is perhaps *the* bird to see on a day in the field. Following the crash in peregrine populations due to organochlorines, work on captive breeding as a conservation technique was developed in the United States by Dr Tom Cade, Professor of Ornithology at Cornell. Now, in **Falcons of the World** (Collins/Cornell University Press; £15, \$38.50), he summarizes present knowledge not only of peregrines but of the entire falcon genus. The first quarter of the book is a general review of falcon biology and ecology, the bulk of the text comprising essays on each of the 39 species with handsome plates by David Digby, whose double spread of a gyrfalcon striking at a ptarmigan is stunning. Though a glossy, large-format work, this is not to be dismissed as a coffee table volume: it is a

scholarly and appealing account of a group of birds which, as Cade says, "more than any other combines form and function in a perfection that can hold men in spellbound wonder".

Though birders have become increasingly well-travelled in the past decade, most would be hard pressed to know where to look for cotingas. The family, which includes bell-birds, umbrella birds and the cock of the rock (ah! now you know) contains about 65 species, mostly specialized fruit eaters, from the forests of South and Central America. In **The Cotingas** (Oxford University Press/Cornell University Press; £30, \$45) David Snow attempts to provide the first comprehensive account of the group, drawing on his own studies as well as on the work of others. Though hardly likely to appeal to a wide readership, the book is certainly of interest to those studying avian courtship behaviour and, as the author sadly remarks,

if it stimulates further fieldwork and encourages wider interest in a family of birds that suddenly, in the last thirty years, after millions of years of evolution, are as threatened as the forests in which they have evolved, it will have achieved another of its purposes.

Closer to home, there is concern for a number of species which show recent declines. One is the barn owl and it is opportune that a major monograph has now appeared. **The Barn Owl** by D. Bunn, A. Warburton and R. Wilson (Poyser/Buteo; £12.60, \$32.50), is based on the equivalent of 38 years of field work between 1963 and 1982, covering virtually all the habitat types used by the species in Britain. The authors' findings are compared with those of other workers world-wide — for the barn owl is perhaps the most widespread land bird in the world. A readable style, good presentation and illustration make the book a model of its type. Once again, ornithology owes a debt to the amateurs who "lose inordinate amounts of sleep . . . get eaten alive by a varied assortment of biting flies . . . and spend long hours when absolutely nothing happens".

It was amateurs too who undertook the three years' field work which produced in the mid-1970s the Register of Ornithological Sites. This ambitious project, run by the British Trust for Ornithology, sought to identify and evaluate as many as possible of the ornithologically important sites in the United Kingdom. Inevitably, it fell short of comprehensive coverage but it has provided conservation planners with a valuable aid for individual site protection, a yardstick and methodology for site evaluation, a pointer to habitat types or areas requiring further coverage and, last but not least, a basic evaluation of the overall resource now published as **Bird**

Habitats in Britain (Poyser/Buteo; £13, \$35). The author, Rob Fuller, was responsible for organization of the national survey and he has drawn not only on its results but on many other sources to describe the composition of bird communities in different habitat types: he also comments on the pressures from current land-uses, notably agriculture and forestry which are the major modifiers of habitats and thus the greatest threat to a number of British species.

A mapping exercise of a different character is represented by Colin Harrison's **Atlas of the Birds of the Western Palearctic** (Collins/Princeton University Press; £12.95, \$25) which shows the summer, winter and resident distribution of all species normally occurring in Europe, North Africa and the Middle East. The text gives accounts of each species' status, climatic range, typical habitats and sites and there are pleasant, if rather irrelevant, half-tone drawings of the species concerned on each page. The layout and presentation are clear and the work provides a partial up-date of Voous' masterpiece, *Atlas of European Birds*, now 20 years old. Perhaps inevitably in such a complex exercise, there are mapping errors but, used with care, the book will be of value, not least to the select band of birders who seek to pursue their hobby wherever birds themselves go. □

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Africa's avifauna

C.M. Perrins

The Birds of Africa, Vol. 1. By Leslie H. Brown, Emil K. Urban and Kenneth Newman. Pp.536. ISBN 0-12-137301-0. (Academic: 1982.) £53.40, \$99.

NO WORK covering the birds of the whole of Africa has been published for over 20 years. *The Birds of Africa* is intended to remedy this state of affairs, and when completed will comprise at least five volumes (with the possibility of a further one to include the birds of Malagasy and the Indian Ocean).

This, the first volume, deals with 10 orders of non-passerine birds from ostriches to birds of prey, some 270 species in all. It starts with 31 useful pages of general information on the geology, climate, geography and habitats of Africa, and with an introduction to the avifauna, the number of species involved and their migrations.

In the main part of the book the information given for each species is fairly standard handbook material, with details

of range and status, description, field characters, voice, general and breeding habits and food, together with a distribution map and one or more pictures on the colour plates. On average some 1½ pages is allotted to each bird, though a few well-known species (such as the ostrich) get as many as five pages and a few of the vagrants only a few lines. Throughout the book the writing is clear and informative, and emphasizes field biology, especially ecological and behavioural studies, the latter often including line-drawings to amplify the text.

Although the standard of presentation is high, there are some shortcomings. For instance the distribution maps raise a few problems. Many in other handbooks show summer and winter ranges. This is more difficult when dealing with African birds because different areas experience such very different seasons. Ideally, however, it ought to be possible to depict breeding and non-breeding distributions and it is a little disappointing that this has not been done, except in a very few cases. In addition there is some inconsistency — albeit minor — in the treatment of distributions of the birds of offshore islands and in mapping bird distributions in Malagasy.

Bird books often stand or fall by the quality of their illustrations, but here the plates — by Peter Hayman and Martin Woodcock — are well up to standard. Some of the paintings are on the small side; in the extreme case there are over 70 birds on a single page. However on the whole they are acceptable because of the large format of the book, and as a group they succeed remarkably well in being both accurate and aesthetically pleasing. For most species there are at least two pictures showing the different sexes or summer and winter plumages; in addition there is often a painting of a young bird.

One minor worry concerned the amount of space available for each species. The dust-jacket states that there will be a further three volumes. However, this first volume includes only about 270 of the 1,850 or so species recorded in Africa. Hence the remaining three volumes would have had to cover 1,600 species, or twice as many per book as Vol.1. Even granted that this volume contains many of the better-known birds, such as the ducks and birds of prey, this would have been difficult. Fortunately the publishers have recently decided that there will be at least one further volume; this will ease things considerably.

The Birds of Africa, an immense project, was the brain-child of Leslie Brown who sadly died before this volume was completed. One very much hopes that the other authors will maintain his drive since the completed work will be a valuable contribution to ornithology. □

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A world of far away and long ago

Andrew Hill

The Historia Naturalis of Pliny the Elder. By Joyce Irene Whalley. Pp.46. ISBN 0-283-98905-X. (Sidgwick & Jackson: 1982.) £6.95.

PROPPED between two slaves poor Pliny died, while the pumice of Vesuvius fell about him. It was his constant search for facts that led to this early demise, a compulsion that had produced a number of books on a range of subjects, now all lost but for the encyclopaedic *Historia Naturalis*. This work justly purported to encompass "... the world of Nature, or, in other words, Life ...", and assembled what Pliny calculated as 20,000 facts, culled from 2,000 other books and his own experience. Including places, peoples, animals, plants, geology, medicine, painting, it provides a rich view of the world in the first century AD. This is a significant view, for it was one that with all its faults and pseudodoxies dominated Europe through the Middle Ages and extended its influence to more recent times.

Joyce Whalley's fascinating work is rather less ambitious, being intended primarily as a picture book. In the 1460s Giuliano Amedei illustrated a manuscript of Pliny with elaborate historiated initials for each of the 37 books or chapters. These depicted scenes related to the subject matter, and occasionally are as fanciful as parts of the text. One, for instance, shows a varied assemblage of humanoids, including the rare umbrella-foot tribe who, disposing themselves upon their backs, avoid the sun's glare through the protection afforded by the shadows of their substantial feet.

Some of these beautiful illustrations, highly ornamented in white vine decoration, resemble secular aspects of the contemporary books of hours, being lively and realistic treatments of everyday fifteenth-century activities. Many also reflect Pliny's patchy accuracy: elephants have lions' feet, and even more readily observable insects have eight or more legs.

Amedei comments visually on Pliny; Joyce Whalley comments on them both. Short descriptive sections opposite each plate indicate their significance, giving a necessarily brief account of the appropriate chapter and selecting phrases, practices and beliefs that have some particularly modern association. This pleasant and enjoyable book provides a vivid and instructive glimpse of the world through earlier eyes. It is also an enticement to explore in more detail the complete work, forming a gentle introduction to the cosmos and nature of Pliny. □

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Creatures of fact, creatures of myth from the sixteenth century

Bernard Wood

Ambroise Paré: On Monsters and Marvels. Translated and introduced by Janis L. Pallister. Pp.224. ISBN 0-226-64562-2. (University of Chicago Press: 1982.) \$20, £14.

IF ANY man has claim to be regarded as the Father of Modern Surgery, it is Ambroise Paré, the greatest of the Renaissance surgeons. Born of a humble family in 1510, Paré became apprenticed as a barber-surgeon, eventually moving to Paris in 1533. He gradually freed himself from his hair-cutting duties and was appointed *aide-chirurgien* at the Hôtel-Dieu, and by the age of 30 had qualified as a barber-surgeon.

Paré's training was essentially practical rather than scholastic; like many distinguished surgeons, he learned much of his craft on the battlefield. His first experience of war was the attack on Turin by Francis I in 1537. He quickly put his experience at the Hôtel-Dieu to good use, and concluded that the application of boiling oil frequently impeded rather than facilitated the healing of missile wounds. His campaigning days ended in 1559, but from then until his death in 1590 Paré managed to remain close to the crown and

Of all his legacies, however, perhaps the richest is the breath, wisdom and humanity of his writings. He wrote not in Latin, but in Old French, and his willingness to discuss any matter on which he had practical experience did not always endear him to his peers. The 1575 edition of his collected works particularly outraged Estienne Gourmelen, the Dean of the Faculty of Physicians in Paris. Paré's reply to Gourmelen was trenchant and typical: "Dare you teach me Surgery, you who have never come out of your study? Surgery is learned by the eye and the hands. You mon petit maître, know nothing else but how to chatter in a Chair".

It is an indication of Paré's prodigious energy that *Des Montres et Prodiges*, first published in 1573, was the nineteenth in his series of surgical writings. Though the work had earlier been translated into Latin and thence into English, this edition is the first to be prepared by translating the original French into English. Janis Pallister's translation preserves the earthiness of the medical literature of the time — the directness and freshness of Paré's prose is in clear contrast to that used in modern-day texts where a leaden style is too often mistaken for scholarship. The dust-jacket claims that the edition includes a scientific commentary. It is true that a physician and a biologist provide notes and suggest identifications or diagnoses of what Paré describes, but these are modest and often merely expand on an earlier commentary by Paul Delaunay.

On Monsters and Marvels, a delightfully whimsical book, is divided into four parts. The first (and much the largest) section is devoted to human and animal monstrosities. Some of these animal "monsters" are descriptions and reports of creatures which are recognizable; others are clearly creatures of the imagination. The first chapters, devoted to human congenital and birth defects, make fascinating reading when one considers the period when the book was written. They include a description of what appears to be an example of the testicular feminization syndrome, and picturesque accounts of malingering and patients suffering from calculi.

Flying, terrestrial and celestial monsters occupy the last three chapters. The "flying" monster turns out to be the ostrich and the terrestrial one the elephant, and the structure and habits of both animals are described and discussed: the observations on the ostrich were based on a skeleton of one of three ostriches kept by King Charles. The suggested identifications and scientific notes are useful but not infallible. For example a stone "under the tongue" of Captain Augustin, Engineer to the King, was almost certainly in the duct of the submandibular gland and

not in the duct of the parotid as is claimed in the commentary.

It may seem to us that a book which dwells on monsters and marvels was an idiosyncratic choice for a surgeon. However in Paré's time such books were in vogue, and we must be grateful to Janis Pallister for making this curious and beguiling work more widely available. □

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Fragments of the past

John Sutton

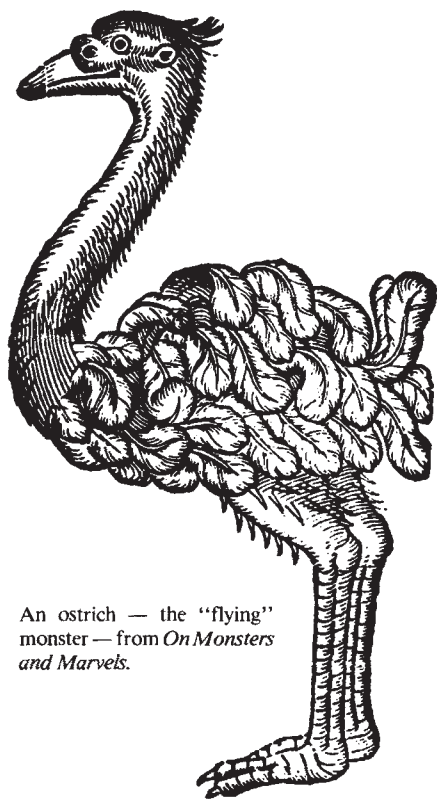
A Geological Miscellany. Compiled by G.Y. Craig and E. Jean Jones. Pp.216. ISBN 0-946193-00-2. (Orbital Press, PO Box 141, Oxford: 1982.) £8.95.

FOR many years it was the pleasant custom of the Geological Society of London to present new Fellows with Woodward's history of the Society's first hundred years. I know of no other book which conveys so effectively the liveliness and fun of those who set geology on its way early last century. Babbage in 1830 wrote of the discussions at the Society that "to say . . . they are very entertaining is the least part of the praise which is due to them".

To my mind Professor Craig and Mrs Jones might have done more to bring out the ability of so many pioneers to do serious work in a light-hearted and enjoyable way, and to convey the excitement of a youthful science. True, they have collected some nuggets, but the grade of the ore they have worked varies greatly and some of the longer of the 130 or so passages in this miscellany might have been consigned to the tip heap in favour of better material.

The two editors nevertheless should be congratulated on their happy thought of bringing together "a pot pourri of adventure, anecdote, epigram, autobiography, discovery, hypothesis and bureaucratic absurdity". There is plenty of material available, for geologists are gregarious people who pass on good tales from one generation to the next: it is still possible to encounter Fellows of the Geological Society who remember Archibald Geikie, who had been out in the field with a geologist who in turn had been on a geological excursion with James Hutton, born in 1726.

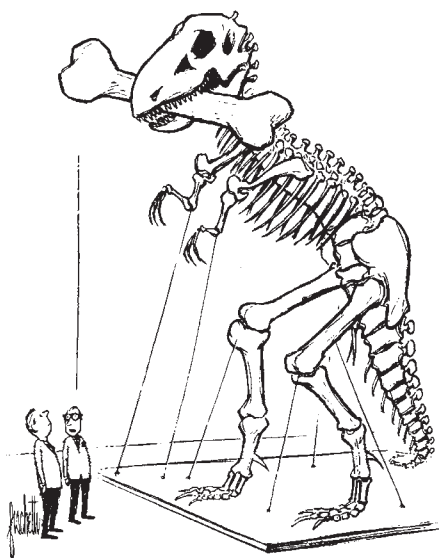
Craig and Jones have cast widely over the English-speaking world from New Zealand to New England and even beyond to Pliny on the destruction of Pompeii.



An ostrich — the "flying" monster — from *On Monsters and Marvels*.

true to both the art and science of surgery. He advanced treatments as diverse as those for hernia and bone fracture, and devised enduring instruments such as forceps for clipping arteries.

Among the treasures they have come up with are verses by J. Maynard Smith on the "Danger of Being too Clever" (part of a collection presented to J.B.S. Haldane) and a poem by Edward Forbes addressed to a lady whom he must subsequently have left for another. We encounter Edgeworth David suspended over an Antarctic crevasse yet too polite to disturb his only companion, Mawson, who was changing photographic plates unaware of Edgeworth David's predicament. We hear of Ami Boué declining an offer of six



"It seemed logical. We had one bone left over."

Bulgarian maidens when in the field early last century, and of Mary Anning's exchanges with the King of Saxony. Mention of royalty brings to mind one of the best geological passages, an account by Queen Victoria of the parallel roads of Glen Roy.

These are good historical vignettes; and there is genuine scientific interest in a letter from Sedgwick to Darwin expressing his reservations on the *Origin of Species*, and in an equally sceptical reaction from a Geological Society of America audience to Griggs's paper on convection currents in the mantle. More as good as these would have been preferable to the accounts of swindlers in nineteenth-century USA, of an alcoholic drive through New Mexico and of diamond hoaxes in England which together take up a tenth of the book. And it was insensitive to guy Edward Greenly's tribute to his wife.

All in all, my verdict is — a good idea, could have been carried out more effectively. Let Johnson (quoted p.161) have the last word. Boswell: "Is not the Giants' Causeway worth seeing?". Johnson: "Worth seeing? Yes, but not worth going to see". That sums up the book. □

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More of dinosaur extinction, and more

Malcolm C. McKenna

Hunting the Past: Fossils, Rocks, Tracks and Trails — The Search for the Origin of Life. By L.B. Halstead. Pp.208. ISBN 0-241-10899-3. (Hamish Hamilton/Double-day: 1982.) £10.95, \$19.95.

BEVERLY Halstead is probably most familiar to readers of *Nature* as the instigator of various criticisms of scientific work at the British Museum (Natural History) and of its epistemologically exciting exhibition policies. Polemics apart, he has also published some very important research on Palaeozoic fishes and on vertebrate hard tissues.

In *Hunting the Past* he shows us some of the wonders of geology, palaeontology and physical anthropology, explained simply and with apt and captivating illustrations, most of which are in colour. The book is aimed at beginners but focuses more on geology and on the romance and history of fossil hunting than on how scientific studies are conducted once the field-work stage is finished. *Hunting the Past* is in the *Time-Life* tradition, a book for the intelligent layman but not for the edification of advanced amateurs or professionals.

Much emphasis is given to the ever-popular dinosaurs. The sections on speed and body temperature are welcome improvements over most of the older popular treatments, in view of the obvious complexity of the subject and Halstead's need to simplify in the space available. At several places in the book, however, the reptile-like mammals (therapsids) are dubbed paramammals, an unnecessary redundancy. One wonders why the dinosaurs were not dubbed parabirds!

On the subject of dinosaur extinction, Halstead discounts the currently fashionable asteroid impact hypothesis, noting correctly that many dinosaurs were already extinct by the time of the Cretaceous-Tertiary impact event and its associated iridium anomaly and disruption of the calcareous marine biosphere. No one doubts that crashing asteroids would have had no effect on already extinct organisms, but the argument that the extinction of the very last of the dinosaurs is close enough to the K-T boundary to coincide with it is now being championed with renewed statistical evidence by Louis Alvarez.

Further, anti-asteroid arguments that dinosaur extinction was diachronous from north to south rest heavily on palaeomagnetic polarity stratigraphy that is itself suspect. Anomalies may be missing from the stratigraphic record of the San Juan Basin of New Mexico because of a mistaken lack of notice given to a late Cretaceous to early Palaeocene unconformity beneath the restricted Ojo Alamo Member of the Nacimiento Formation. Moreover, Archibald recently reported a lignite ("Will the Real Z Coal Stand Up")

beneath the famous Bug Creek Anthills site in Montana. There are even rumblings that dinosaurs have been found above what is identified as the Z coal elsewhere. The fat is likely to remain in the fire for some time while all this confusion is sorted out. Meanwhile, Halstead is unwise to play down the asteroid impact hypothesis as a cause of extinction of the last of the parabirds.

In the anthropological part of the book the Piltdown Hoax caught my eye. Even the Loch Ness Monster and Glen Rose Cretaceous "human" footprints from Texas get space. And of course there are some amusing errors and misprints, such as Powell supposedly discovering the Grand Canyon, the multituberculates being categorized as rodents or the apt description of Glen Rose footprint modifiers as "Cretationists".

At the end of the book there is a section on classification in which, true to form, Halstead misinterprets cladistics. He seems to have read Hennig's 1966 book, but the subject has progressed a bit since then. In all, Halstead's attempt to purvey palaeontology and geology to a non-professional audience is a creditable effort — but it falls far short of excellence. □

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Going to ruins in central America

Warwick Bray

The Complete Visitor's Guide to Mesoamerican Ruins. By Joyce Kelly. Pp.527. ISBN 0-8061-1566-1. (University of Oklahoma Press: 1982.) \$35.

THERE have been, as the saying goes, plenty of guide books to Mesoamerican ruins, but never one like this. The author covers 119 archaeological sites (graded from one to four stars, in terms of viewability) and also 41 museums in Mexico, Guatemala, Belize, Honduras and El Salvador. A "Complete Guide" it is not, and every reviewer will lament different omissions (I particularly regret the Cacaxtla murals and the new excavations at the Aztec Templo Mayor in Mexico City), but all the principal sites are included, as well as an enterprising selection of second quality sites in the Maya zone and Pacific Guatemala.

Information on the cultural background to the architecture is rather sketchy, and technical terms are occasionally used

without explanation. On the credit side, the book is lavishly illustrated with photographs, but would benefit from more detailed site maps, and has only a short and selective bibliography at the end. For each individual site the author includes a section (sometimes quite lengthy) on the history of discovery and research, giving the names of the excavators, with their institutional affiliations and the dates of their investigations, but not the full bibliographical references that would carry the serious amateur forward to the next level of reading. This merely causes frustration, and was a serious error of judgement on the part of the author or publisher.

The introductory chapter contains much good general advice, on everything from camera equipment to car insurance, but is strongly slanted towards the North American reader. British tourists should check their own visa requirements and, with the collapse of the Mexican peso, should watch out for changes in currency regulations. Probably, too, El Salvador is a place to keep away from at the moment.

The emphasis throughout this book is on how to get to the sites and what to see there. The "ruin buff" (a favourite phrase of the author) to whom this advice is directed comes over as rather timid and of a certain age, driving his — or more probably, her — own car, worried about snakes, insects, sunburn and where to find the nearest cold drink, and in a perpetual dither about whether to wear boots or tennis shoes. Carrying a book of this size, too big and heavy to fit the pocket, walking is going to be hard work. Eye make-up tends to run, but if it rains you can always "carry a wig to wear when you get back to the cities or until you can get to a beauty shop". The main purpose of the visit is not to study aboriginal culture, but to get the best possible photos (or, at least, the same ones that everybody else takes) in the shortest possible time.

It's easy to poke fun, but this is a perfectly legitimate market to aim at. As a visit to any major ruin will demonstrate, there are a lot of people just like this (so many that they tend to get in the way of each others' photos), and they very often need precisely the kind of help that Joyce Kelly's book provides. So, too, do the more snobbish, those of us who through professional arrogance or sheer carelessness have got lost, gone hungry or arrived at a site to find the main buildings in deepest shadow. Thanks to Mrs Kelly, we should not make those mistakes again.

Warwick Bray is Reader in Latin American Archaeology at the Institute of Archaeology, University of London.

Review supplements for 1983

Dates of the five special review issues to be published by *Nature* in 1983 are: Textbooks, March 10th; Spring Books, April 28th; Journals, October 6th; Autumn Books, November 10th; and Christmas Books, December 1st.

Wiggles put straight

Peter J. Smith

Inside the Earth: Evidence from Earthquakes. By Bruce A. Bolt. Pp.191. Hbk ISBN 0-7167-1359-4; pbk ISBN 0-7167-1360-8. (W.H. Freeman: 1982.) Hbk \$22.50, £17.80; pbk \$11.95, £9.95.

PROFESSOR Bolt has dedicated his latest book not only to the pioneers of seismology in general but to Richard Dixon Oldham in particular, and with good reason. Though little remembered these days, it was Oldham who first showed, in 1906, that earthquake waves could be used to demonstrate the existence in the Earth of major physical discontinuities and thus of a large distinct central core. Indeed, the reading of Oldham's paper on the subject to the Geological Society was a far more significant event for the Earth sciences than the now better-known San Francisco earthquake that was to follow just a few weeks later, for although the disaster in California undoubtedly gave both a scientific and a political boost to the study of seismology, it was Oldham who enabled such study at last to be "removed from the realm of speculation into that of knowledge".

The particular dedication is also appropriate in a quite different sense, even if Professor Bolt would not say so himself. Oldham's 1906 paper was a model of exposition; and in his own way and with far less tractable material Professor Bolt hardly achieves less. There are, for example, few things in seismology more difficult to explain to the non-specialist or undergraduate than terrestrial spectroscopy and few things more mind-boggling than trying to envisage the overtones of the spheroidal and toroidal eigenvibrations of the Earth. After reading Professor Bolt's 18-page chapter on these free oscillations, many may well feel that some of the minor mathematical and notational details elude them; but they will hardly come away with less than a very firm grasp of the physical principles involved. To be able to impart a strong sense of what is actually going on, as opposed to merely presenting some obscure mathematical description, is no mean achievement.

On the principle that anyone who can successfully explain the nature of the Earth's free oscillations can explain anything, it comes as no surprise to find that Professor Bolt maintains the same high standard of exposition in dealing with the rather less exotic, but no less interesting, aspects of seismology — the nature and measurement of earthquake waves, the significance of wave paths and travel times, the interpretation of seismograms in terms of broad Earth (and lunar and Martian) structure, the elucidation of finer (transition zone) structure, the estimation of density from seismic properties and so on.

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The result is a brilliant non-specialist introduction to the ways in which "the wiggles carry news about the deep interior of the Earth". It can be used as an elementary text in seismology, as a primer for non-seismological Earth scientists or by the layman who seeks a lucid account of the subject; for each of these audiences, not the least of its strengths is that it takes

trouble to explain those fundamental principles and ideas (for example, the nature of direct and inverse problems) that so many authors of even basic texts mistakenly take for granted. □

Peter J. Smith is Reader in the Department of Earth Sciences at the Open University, Milton Keynes, and editor of Open Earth.

Carbon dioxide and climate (and journalism)

John S. Perry

Future Weather and the Greenhouse Effect. By John Gribbin. Pp.302. ISBN 0-440-02498-6. (Delacorte Press/Eleanor Friede: 1982.) \$15.95.

JOHN GRIBBIN essays a formidable task: to explain the genesis, mechanisms, history and probable future course of Earth's climate in some 300 pages of non-technical prose. His presentation is admirable in its design and broad thrust, but he has left some nasty booby traps for the unwary reader.

Over the lifetime of our planet, atmosphere, climate and life have evolved together. Climate has varied throughout the long history of the globe, and the short history of man. Rapid changes in climate could have drastic consequences and are a

legitimate cause for concern in a world already uncomfortably dependent on crops from a few climatically favoured lands. The most important human influence on climate is likely to be an increase in atmospheric carbon dioxide from the combustion of fossil fuels; models of the Earth's atmospheric and oceanic systems project significant changes in the workings of the great heat engine that powers our winds and governs our climate. Future weather will be different, and mostly warmer.

However carbon dioxide "feeds" plants, farmers are clever and people will probably continue to starve or grow fat primarily because of society's aberrations, not those of the weather. Changing climate — whatever its origin — simply complicates our task of providing energy, food and a decent life for everyone on Earth.

Communication of this thread of understanding to the public would be a noble achievement. Indeed, Gribbin's attempt has certain points to recommend it. He wields a breezy, conversational style and has a journalist's eye for arresting details of personality and event. Moreover, his explanations of certain scientific concepts and research results are outstandingly clear and accurate. For example, his discussion of the greenhouse effect is a model of its kind, and the core of his outline of the general circulation is probably as good as can be achieved without recourse to mathematics.

Unfortunately, other flaws sadly vitiate the book's value and betray its promise. The introductory chapters are crammed with a motley assortment of climatic titillations — droughts and blizzards and ice ages — each hurriedly and simplistically linked to one or another meteorological abstraction. Jet streams, the circumpolar vortex and the Intertropical Convergence really make sense only in the context of the general circulation, an insight postponed until Chapter 3. By that point, the lay reader may find himself mixed up and turned off.

The major failing seems to stem from a mismatch between the nature of science and the demands of journalism. There is a popular misconception that scientists are people who look for answers. That's nonsense, of course: although they

generally share a working consensus, scientists are at root people who like to ask questions and pose hypotheses. Bearers of all sorts of ideas are eagerly welcomed to science's great and never-ending party, and the climate corner of the affair — one pictures it as lying between the hors d'oeuvres and the bar — is particularly crowded.

Thus, climate vies with politics, economics and religion in the number and variety of theories per unit of fact. All attempt to explain some aspect of the behaviour of a complex and poorly observed heat engine employing turbulent working fluids, subject to changing forcing, and tampered with to an unknown extent. Variability on all time-scales is a natural and inherent characteristic of climate, so it is no wonder that plausible connections can be adduced between almost any hypothesized cause and virtually any climatic consequence. Few survive independent data and scrutiny, yet Gribbin appears ready to embrace almost any theory — one longs to sell him a used car. The Sun-weather relationships, for instance, to which many pages are devoted, have proven particularly fragile, and it is misleading to treat these empirical speculations as established facts. Physics should rule over statistics, and a treatise on climate should seek to present a sound consensus and be built upon critical scientific judgement.

Instead, the book seems to have been constructed with the help of a vacuum cleaner and a glue pot. One imagines the author pointing his nozzle into all the dusty corners of the village library and cheerfully pasting up with unbounded enthusiasm whatever comes down the pipe — trash and treasure alike. One scientist's solar-based forecasts have "invariably proved right", a judgement shared by few and contradicted a page later. The atomic bomb and man-made aerosols are brandished as precursors of an ice age. For some reason, one dubious claim of geomagnetic-climatic connections merits an entire chapter, while another naive misinterpretation of irrelevant observations "commands respect" in assessing CO₂-induced climate changes. It is astonishing that Gribbin, a physicist by trade, swallows an outrageous claim that even an atmosphere totally opaque to thermal radiation could warm the surface by no more than 4°C. Physics aside, hasn't he ever heard of the heat wave on Venus?

Future Weather and the Greenhouse Effect has many ingredients of a badly needed commodity — a well-balanced and comprehensive book on climate for the layman. The man in the street, however, would be best advised to look elsewhere for a balanced understanding of knowledge and speculation is this important and exciting area of science. □

John S. Perry is Executive Secretary to the Board on Atmospheric Sciences and Climate, National Academy of Sciences, Washington DC.

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A universe without mathematics

Hermann Bondi

The Lighter Side of Gravity. By Jayant V. Narlikar. Pp.194. Hbk ISBN 0-7167-1343-8; pbk ISBN 0-7167-1344-6. (W.H. Freeman: 1982.) Hbk \$17.95, £13.95; pbk \$9.95, £6.95.

JAYANT Narlikar's *The Lighter Side of Gravity* is a splendid effort to bring together many of the most exciting facets of physics and astronomy in a logical argumentation devoid of mathematics. Much so-called popular writing on physical science is content with a description of where today's science has got to; moreover, such a description is often marred (particularly in the media) either by delighting in stressing how "crazy and incredible" it all is, or by regarding the development of science as a sort of intellectual stock exchange where individual scientist's stock rises and falls according to how "right" or "wrong" their views have turned out to be (as though a scientist's standing depended on this!).

Narlikar is wholly free from these faults. He respects his reader and takes him gently, almost imperceptibly, through many of the important arguments leading to the advanced concepts of black holes, of the source of stellar energy, of white holes and of the structure of the Universe. He is outstandingly successful in this, though I feel that some of his derivations are a trace old-fashioned in their nature and in the analogies used. His clear writing, stressing how intelligible it all is, is a delight and indeed a breath of fresh air in contrast to so much of the sensation-hungry writing in this field.

There can be no doubt that this book meets an important need and meets it very well indeed. Yet one must admit that it is a curious aberration of our educational system that the need for such a book without mathematics is so great. Mathematics is used in physics and in astronomy to make arguments easier, to make the work lighter. Yet we create such a phobia of equations and formulae in the bulk of the population that there is a continuing demand for books that try to

make the going easier by explicitly *excluding* mathematics. This is no fault of the author or of this splendid book, but rather a fault of us all for engendering in so many thinking people a violent reflex action to anything mathematical. □

Sir Hermann Bondi is Chairman of the Natural Environment Research Council. His books include The Universe at Large (Heinemann, 1961) and Assumptions and Myth in Physical Theory (Cambridge University Press, 1967).

Sky-watchers A to Z

Stephen P. Maran

Observatories of the World. By Siegfried Marx and Werner Pfau. Pp.200. ISBN 0-7137-1191-4. (Blandford/Van Nostrand Reinhold: 1982.) £8.95, \$16.95.

IN *Observatories of the World* two astronomers at the University of Jena tell tales of 40 of their favourite observatories from Abustumani to Zelenchukskaya. The observatories have been selected to represent institutions of different types, including those that are research-orientated and education-orientated, those with optical, radio and infrared instruments or specializing in elaborate computational tasks, and those located in cities, on mountaintops or (in one case) aboard an aircraft. The English-language translation is by Simon Mitton, who also revised the text.

There is no common theme to the text, except perhaps historical development and, in a depressing number of cases, a history of gradually moving the facility further and further away from a growing metropolis. The authors have much to say about the research programmes that characterized the golden eras of the older observatories, but seem to base their occasionally perfunctory accounts of some modern facilities on the replies to

questionnaires that were distributed to the observatory authorities. This is not a professional reference work, then, nor a directory; there are no tabulations of telescopes and instruments, nor of observatory coordinates and altitudes.

Not all of the facilities described would have made my "Top 40"; the omission of Yerkes Observatory (mentioned in passing, and incorrectly, as being located "in the East of the United States") is particularly unfortunate for it was at Yerkes that much of modern astrophysics was born. Cerro Tololo Inter-American Observatory, that great and much-used telescope farm in Chile, is also not included, although it is briefly described in the section on its sister facility at Kitt Peak.

Observatories of the World is written for those who have some familiarity with the terminology of astronomy, although a number of elementary concepts are explained. It is extensively illustrated with attractive half-tones and rather primitive sketches (plates 11 and 12 appear to be interchanged, incidentally, but most readers should be able to tell a coronagraph from another kind of telescope). Only here, among books that come conveniently to mind, can one readily determine that the ancillary building of Konkoly Observatory near Budapest "has a grass-covered roof and cannot therefore be a source of troublesome air turbulence. . ." or that the little town of Coonabarabran provides scientists with "all the comforts of civilization". Amidst the interesting trivia, however, there is a brief but frank discussion of optical problems with the original 6-metre primary of the huge reflector at Zelenchukskaya, and many other informative tidbits.

The overall result is a pleasant and informative book, despite some awkward verbal constructions such as "Every observatory not recently established. . ." (p. 29) where "The older observatories. . ." might have done as well. If you are an armchair astronomer and you like to read about observatories, this is the book to buy. □

Stephen P. Maran is Senior Staff Scientist in the Laboratory for Astronomy and Solar Physics at NASA-Goddard Space Flight Center, Greenbelt, Maryland.



Mount Wilson Observatory in southern California. To the left are three solar telescopes, centre and right are the domes of the reflectors. The picture is taken from Richard Learner's *Astronomy through the Telescope*, recently published by Evans/Van Nostrand Reinhold. Prices are £12.50, \$29.95. The excellent illustrations and accessible but comprehensive text make the book a stimulating guide to the history of astronomy and its instruments for both professional astronomer and layman.

The cosmos and its contents

David W. Hughes

NEW BOOKS on astronomy continue to flood on to the market, the fact that we are not swamped by them being due to their short life; few last more than two years before going out of print or finding their way onto the remainder shelves. The astronomical second edition is indeed a rarity. Nineteen eighty-two, however, does have advantage over other years — it is the twenty-fifth anniversary of the start of the space race. My first book deals with just that.

Sputnik to Space Shuttle by Iain Nicolson (Sidgwick and Jackson, £7.95) chronicles the childhood of the space age. We start with the characteristic “bleep bleep” of the Soviet Union’s Sputnik 1 which astonished the world on 4 October 1957 and end with the graceful landing of Columbia which completed the fourth test of the Space Shuttle in June of this year. Between these dates there is all the excitement of the race into space, the Soviet Union and the United States continually jockeying for the lead. First dog in space, man in space, woman in space and space walk went to the USSR; first voice from space, navigation satellite, meteorological satellite and communications satellite went to the USA; first photographs of the far side of the Moon, first lunar soft-landing, first Venus soft-landing to the USSR; first men on the Moon, first two-planet mission, first Mars lander to the USA. One could continue but why not just read Nicolson’s book. He is a lively writer with a clear, easily readable style and his book is an excellent review of our 25 years “out there”.

For a more scientific bias we have **Planets of Rock and Ice** by Clark R. Chapman (Charles Scribner’s Sons, \$13.95), a revised and expanded edition of his 1977 book *The Inner Planets*. The author folds together our knowledge of terrestrial planets with the new-found information gleaned from the space probe observations of the satellites and rings of Saturn and Jupiter. This book is raised above the mundane plane of the usual account of the planets by the many anecdotal insights into the workings of the mind of a planetologist that grace its pages. Chapman loves his job, loves the planets and it shows.

In the book he inveighs against the shut down of Solar System explorations that has been inflicted on the American scientific community. There is, however, a very minor advantage to this in that scientists will no longer be distracted by the “gee whiz” findings of new planetary encounters or the need to plan for forthcoming missions. So the storerooms of accumulated information, the thousands of pictures and unprocessed magnetic reels of data, now have a chance of being sifted and scrutinized. Be this as it

may, there are still a multitude of places to go to and experiments that cry out to be performed.

The evolution of Earth’s surface and atmosphere and the way it contrasts with its rocky neighbours is obviously of great interest and concern to Chapman. His ability to express this vividly makes the book a joy to read. But how different is the state of planetology today in comparison to the time when Apollo 11 landed. The London bookmakers William Hill were then offering odds of 100:1 against a manned Mars landing before 20 July 1976 and “evens” on a landing before 20 July 1979!

The Planet Venus by Garry E. Hunt and Patrick Moore (Faber & Faber/Faber Inc.; £10, \$22) divides neatly into two parts. Venus before the space age is mainly written by Patrick Moore and concentrates on the way Earth-based telescopic and spectrographic observations moulded our pre-1962 view of the planet. Transits, the Black Drop problem and the ashen light are carefully reviewed, as are more marginal and esoteric phenomena such as the debate that took place in the 1950s as to whether the surface was covered with oil, water or dust, the aphroditographic work of Percival Lowell that “revealed” a series of dark patches and strips on the planet and a rotation period synchronous with the orbital period, the discovery of a phantom satellite and its subsequent demise, and the early attempts at measuring the surface temperature.

Venus made its entry into our space age in December 1962 when Mariner 2 flew by, and the voyages of discovery have continued with a whole series of Venera, Mariner and Pioneer spacecraft. Garry Hunt reviews the latest ideas about Cytherean greenhouse effects, atmospheric sulphur cycles, wind profiles, circulation patterns, and day and night variations. Pictures are shown of the surface of Venus near the Venera 9, 10 and 13 spacecraft, and the results of the radar observations of the landscape using data from the Pioneer orbiter and the Arecibo and Goldstone radar facilities are reviewed.

Venus is often regarded as the Earth’s twin but an identical twin it certainly is not. This book is a very good account of our knowledge of our nearest planetary neighbour; I was especially impressed by the extensive reference section which will encourage readers to delve further.

Now for something completely different. What started in the United Kingdom in 1767 and the United States in 1855? It was the forerunners of the **Astronomical Almanac** and the 1983 version (US Government Printing Office/HMSO; \$16, £9.25) would make an excellent gift for the astronomer who has

nearly everything. Where else would you easily find the universal times of the greatest northern elongation of the satellite of Pluto close by lists of the 194 brightest galaxies (together with diameters, photometric data and velocities), the 1,482 stars brighter than 4.5^m, the latitude and longitude of the world’s optical observatories, the circumstances of all the 1983 eclipses, together with a host of all the usual things we refer to the almanac for? Many happy hours can be spent dipping into this book.

To move closer to the stars, **The Astronomy Handbook** by James Muirden (Kingfisher/Archon; £3.95, \$8.95) is a colourful, profusely illustrated guide to the constellations, Sun and Solar System. It is aimed at the teenage astronomer. Muirden concentrates on the practical side of the subject and I really like his plan for a do-it-yourself planetarium — no young astronomer’s bedroom should be without one. The book is full of encouragement, more than enough to make any youngster grab a pair of binoculars, brave the cold of a starry night and take to examining the heavens.

Planets first, stars next and finally the Universe and all the theories and phenomena of the cosmos. **Deep Space** by Colin Ronan (Macmillan, London/Macmillan, New York; £10.95, \$25.95) and **The Restless Universe** by Nigel Henbest and Heather Couper (George Philip, £8.95) both throw their net wide and try and sweep in the whole zoo of astronomical objects from pulsars, quasars and black holes down to stars and planets. Both delve into the mysteries of the big bang, the search for alien life, how it will end (the big crunch?), and the birth and death of stars. It is the differences between the two books that are really startling. Ronan’s book is a visual treat and is profusely illustrated not only with photographs of celestial objects but also with a host of well-conceived figures: for example, one diagram shows the relative positions of the brightest members of the Local Group of galaxies and with the accompanying table can form the basis of a model; another does the same for the Sun’s nearest 24 stellar neighbours. It is very hard to resist making these models. I gave in straightaway and had great fun. Time and again the illustrations really punch home the message of the text. The figures are exciting, the text interesting and I have no hesitation in recommending Ronan’s book.

Henbest and Couper confine themselves to 24 figures and 24 colour photographs and I feel that the authors have set themselves the age-old problem of describing a spiral staircase with their hands in their pockets. This comment apart it must be admitted that the text rattles along at a heady pace — nobody will fall asleep reading this book. □

David W. Hughes is a Lecturer in Astronomy and Physics at the University of Sheffield.